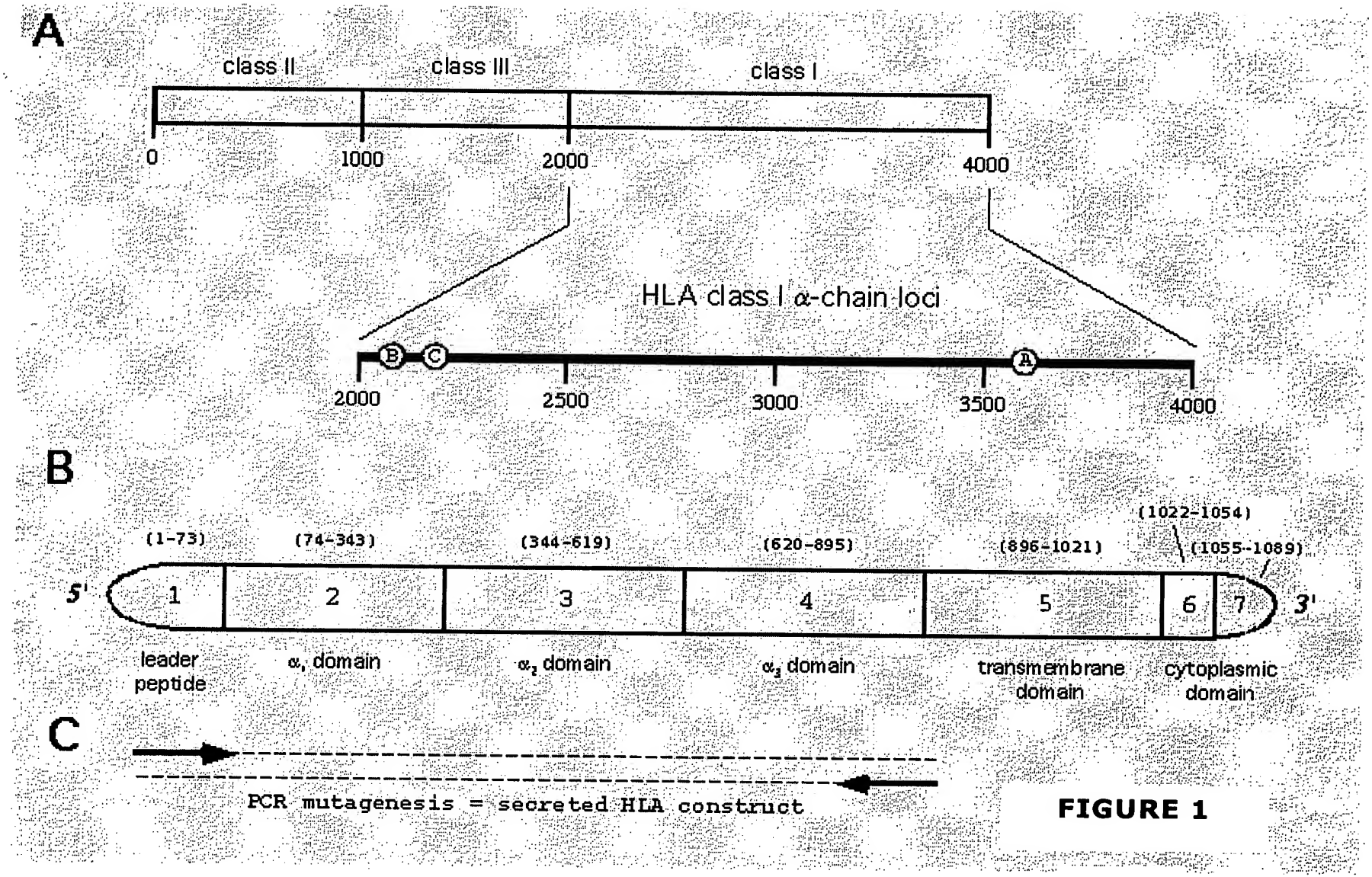
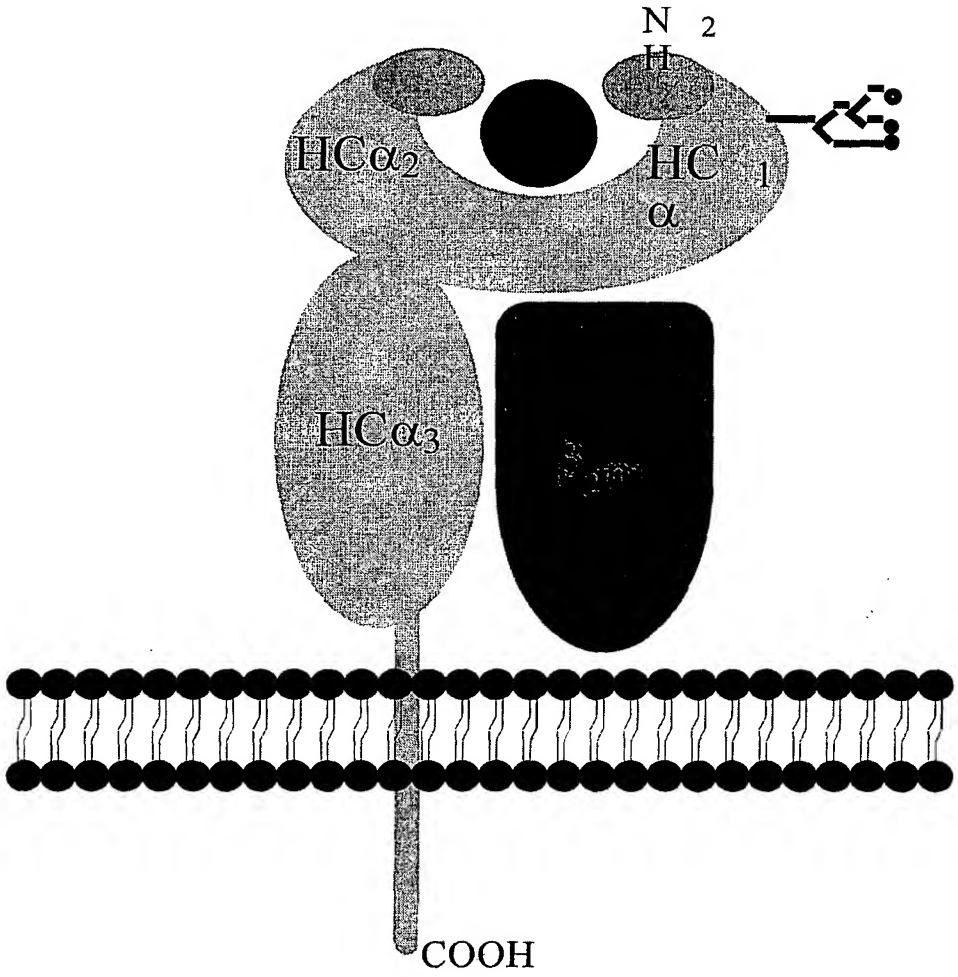
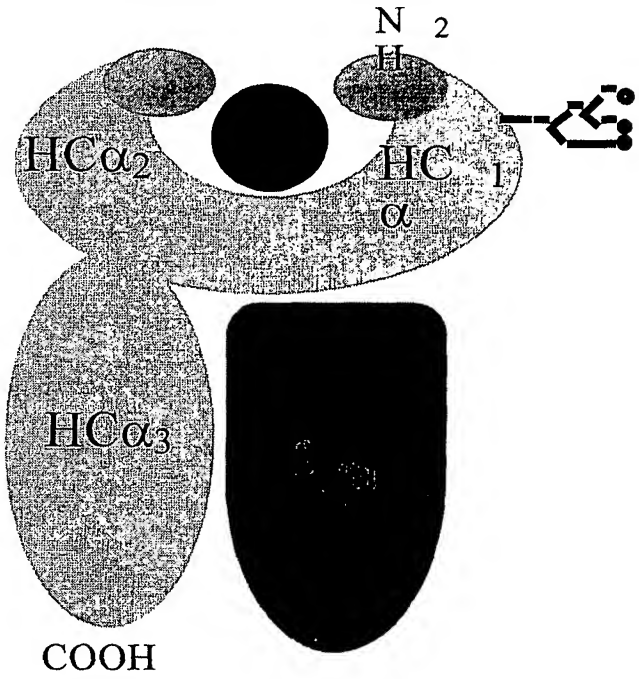

A
class II
class III
class I
0
1000
2000
4000
HLA class I α-chain loci
B
C
A
2000
2500
3000
3500
4000
B
(1-73)
(74-343)
(344-619)
(620-895)
(896-1021)
(1022-1054)
(1055-1089)
5'
1
2
3
4
5
6
7
3'
leader peptide
α1 domain
α2 domain
α3 domain
transmembrane domain
cytoplasmic domain
C
PCR mutagenesis = secreted HLA construct

FIGURE 1



Native, transmembrane HLA molecule



Recombinant, truncated sHLA molecule

FIGURE 2

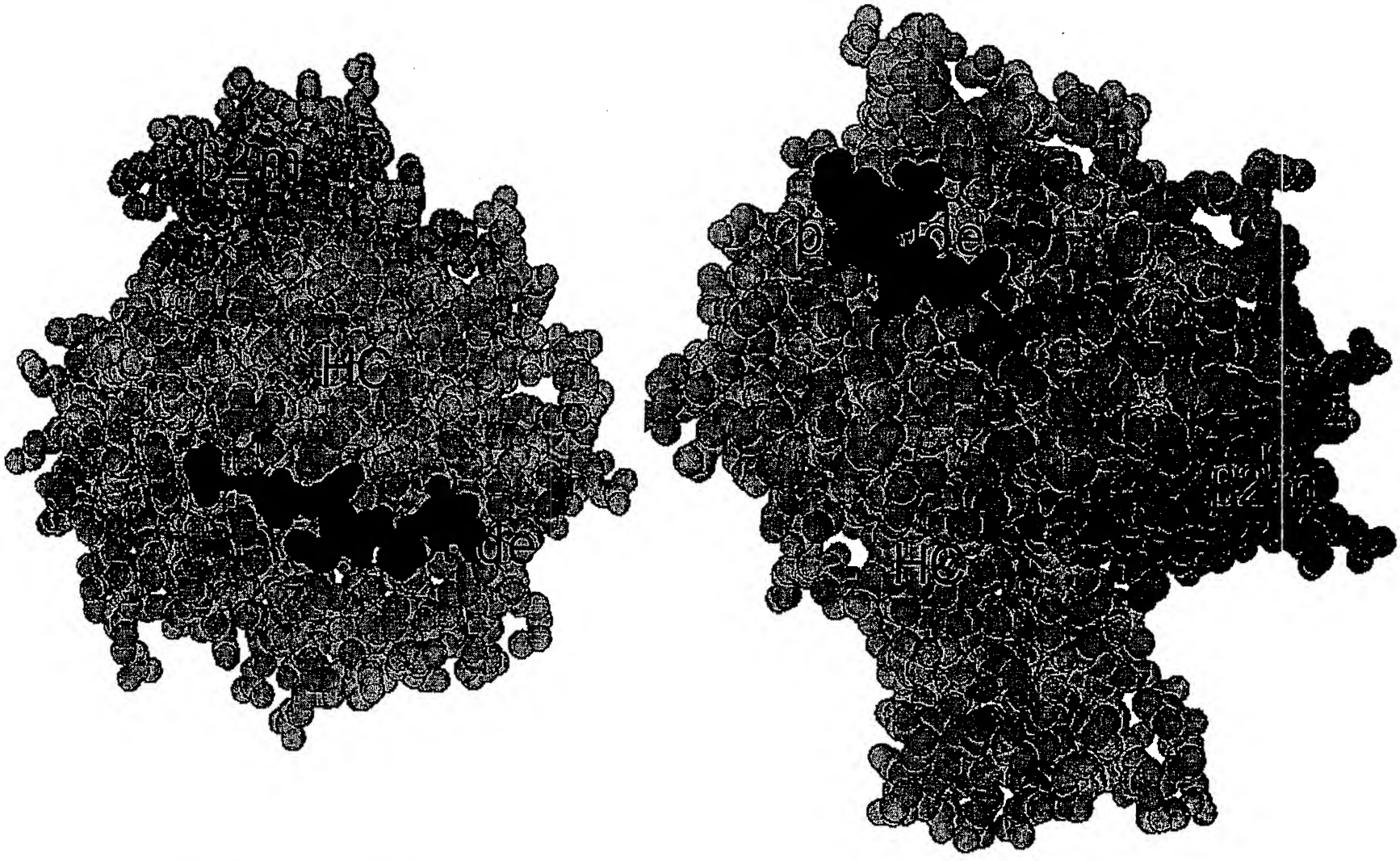


FIGURE 3

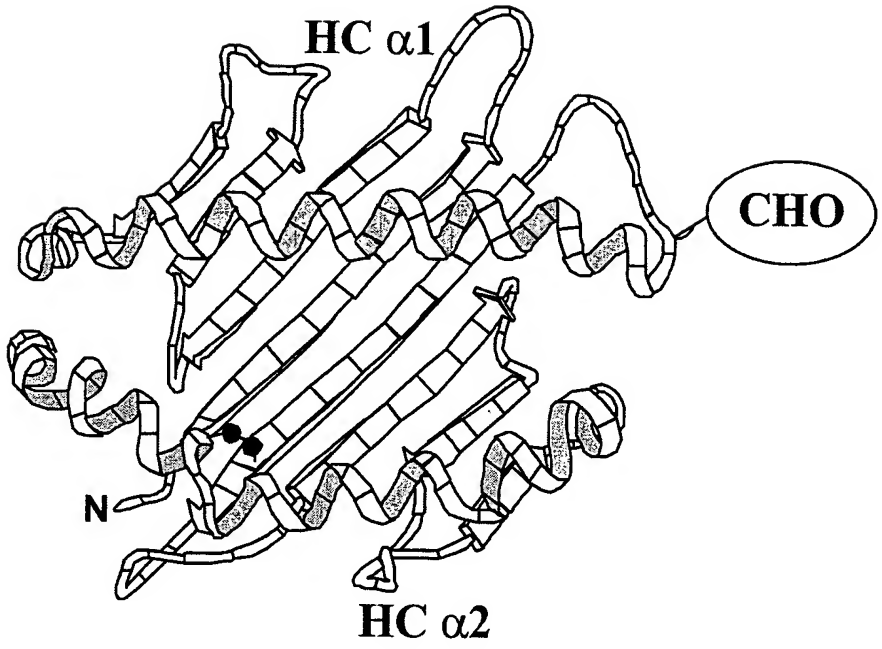


FIGURE 4

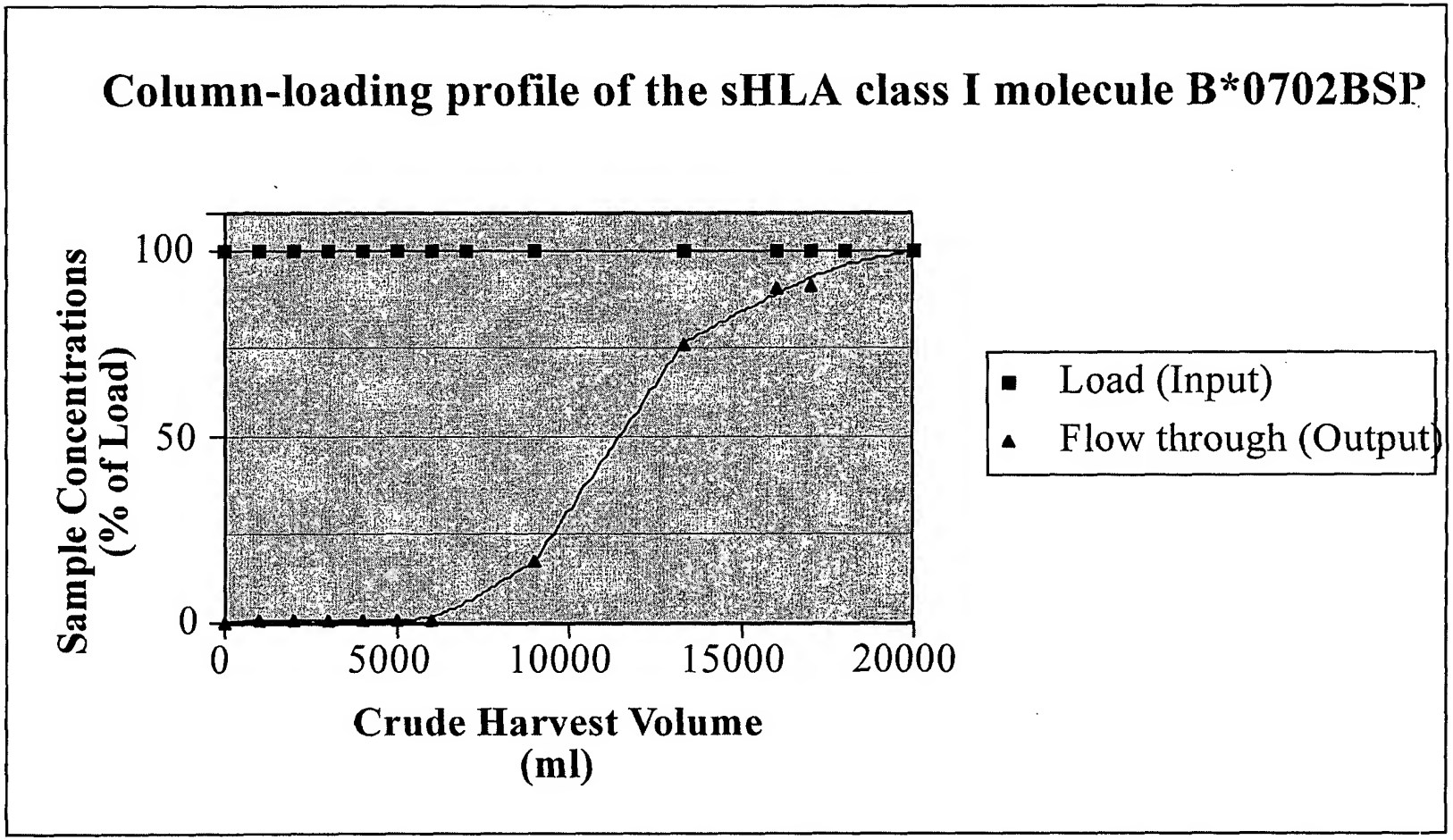
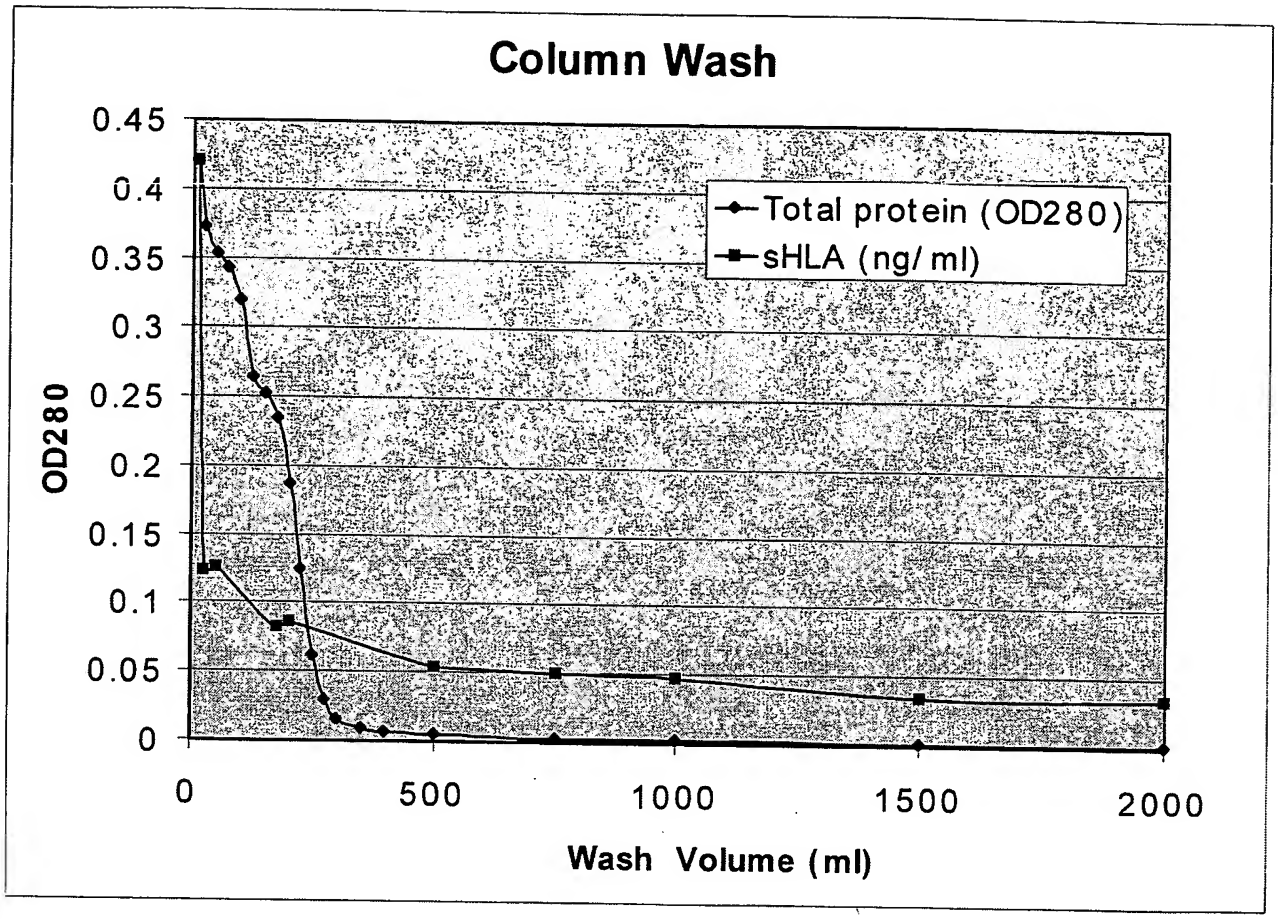

Column-loading profile of the sHLA class I molecule B*0702BSP
Sample Concentrations (% of Load)
100
50
0
0
5000
10000
15000
20000
Crude Harvest Volume (ml)
Load (Input)
Flow through (Output)

FIGURE 5



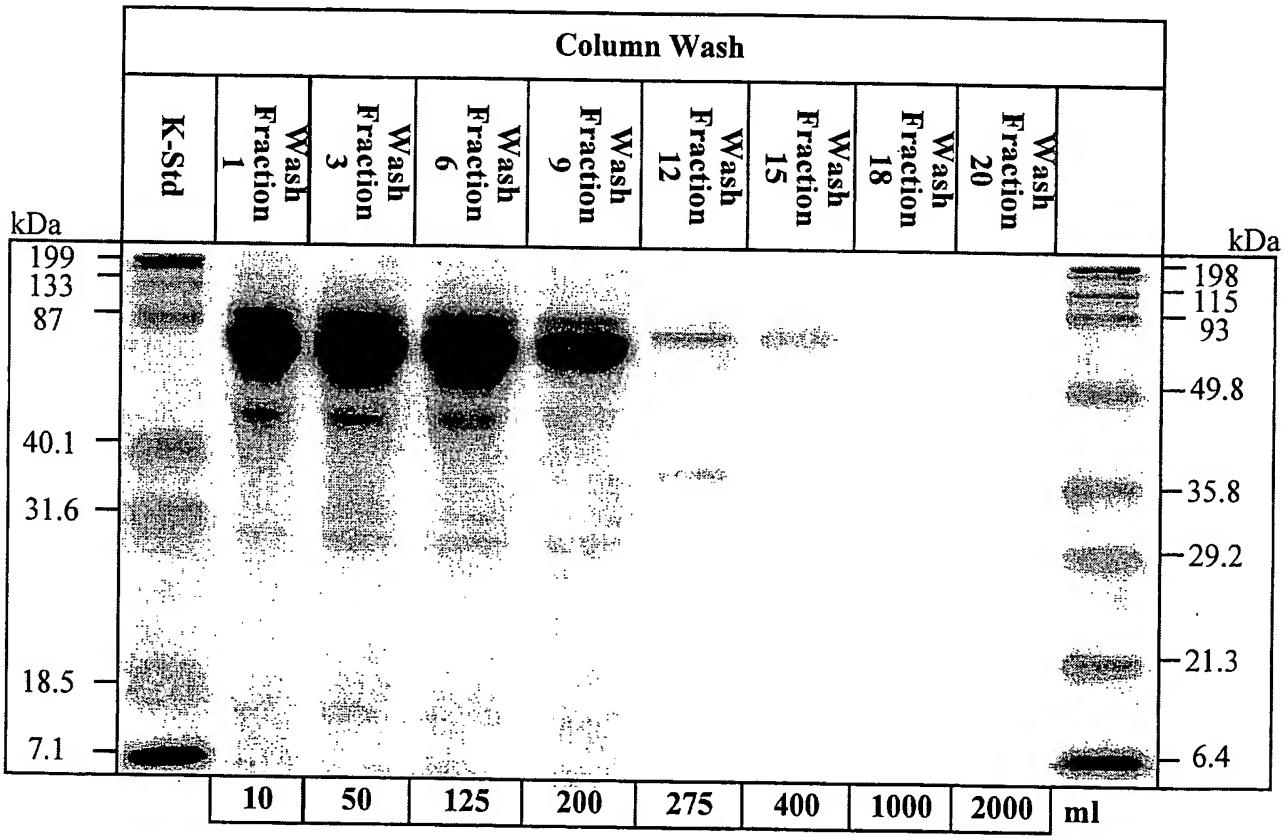
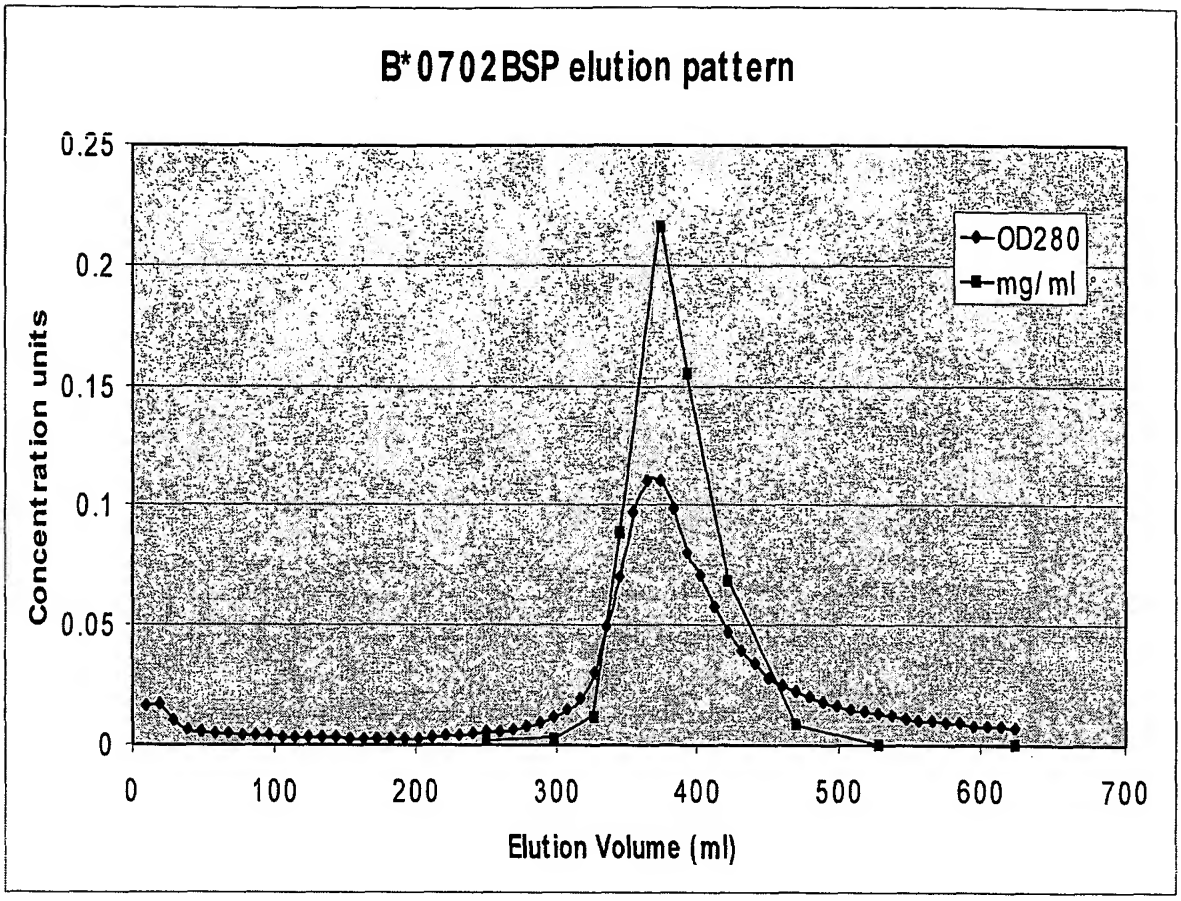


FIGURE 6


B*0702BSP elution pattern
Concentration units
0.25
0.2
0.15
0.1
0.05
0
OD280
mg/ml
0
100
200
300
400
500
600
700
Elution Volume (ml)

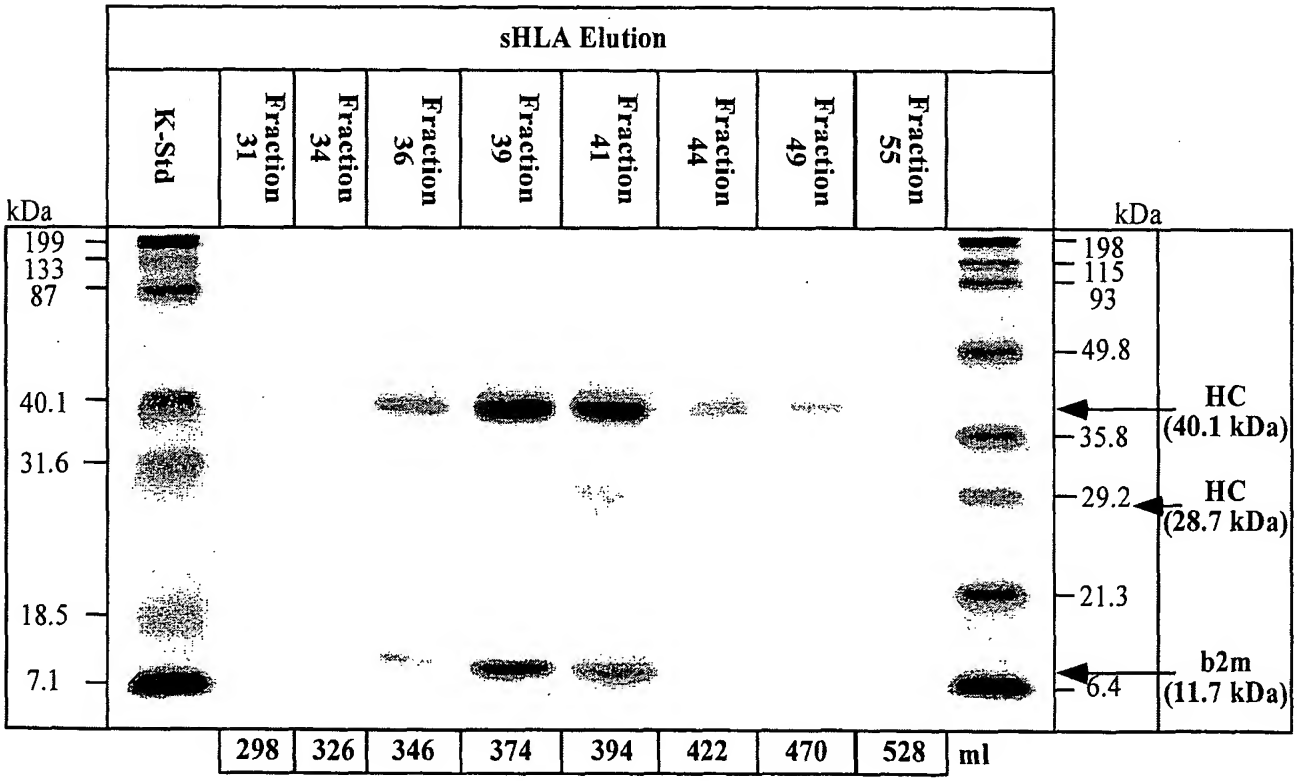

sHLA Elution
K-Std
Fraction 31
Fraction 34
Fraction 36
Fraction 39
Fraction 41
Fraction 44
Fraction 49
Fraction 55
kDa
199
133
87
40.1
31.6
18.5
7.1
kDa
198
115
93
49.8
35.8
29.2
21.3
6.4
HC (40.1 kDa)
HC (28.7 kDa)
b2m (11.7 kDa)
298
326
346
374
394
422
470
528
ml

FIGURE 7

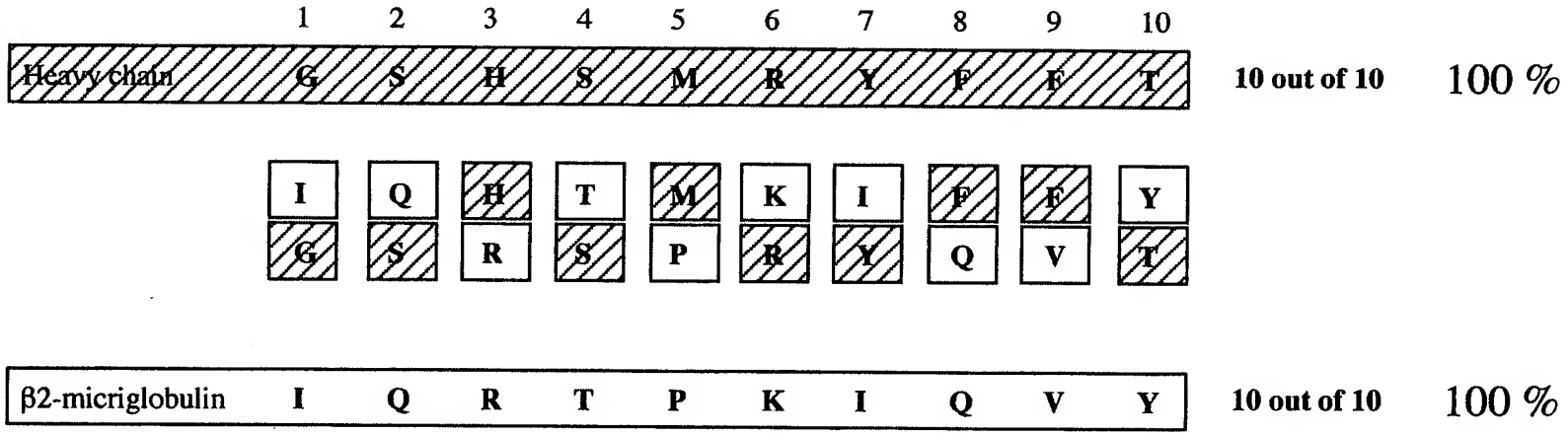

1 2 3 4 5 6 7 8 9 10
Heavy chain G S H S M R Y F E T
10 out of 10
100 %
I Q H T M K I F E Y
G S R S P R Y Q V T
β2-micriglobulin I Q R T P K I Q V Y
10 out of 10
100 %

FIGURE 8

				Calculated residues	Observed residues	Difference
				aa	aa	%
1	A	Ala	Alanine	26	26.2	0.8
2	C	Cys	Cysteine	[6]	-	
3	D + N	Asp+Asn	Aspartic acid + Asparagine	36	34.9	2.9
4	E + Q	Glu+Gln	Glutamic acid + Glutamine	50	47.8	4.3
5	F	Phe	Phenylalanine	11	11.3	2.5
6	G	Gly	Glycine	[23]	-	
7	H	His	Histidine	13	11.4	22.6
8	I	Ile	Isoleucine	12	13.2	9.8
9	K	Lys	Lysine	15	17.9	19.4
10	L	Leu	Leucine	26	28.5	9.5
11	M	Met	Methionine	5	4.7	6.4
12						
13	P	Pro	Proline	20	21.1	5.4
14						
15	R	Arg	Arginine	31	30.5	1.8
16	S	Ser	Serine	23	22.3	2.8
17	T	Thr	Threonine	27	24.6	9.0
18	V	Val	Valine	18	18.6	3.5
19	W	Trp	Tryptophan	[10]	-	
20	Y	Tyr	Tyrosine	20	20.0	0.1
			Total	333	333	

FIGURE 9

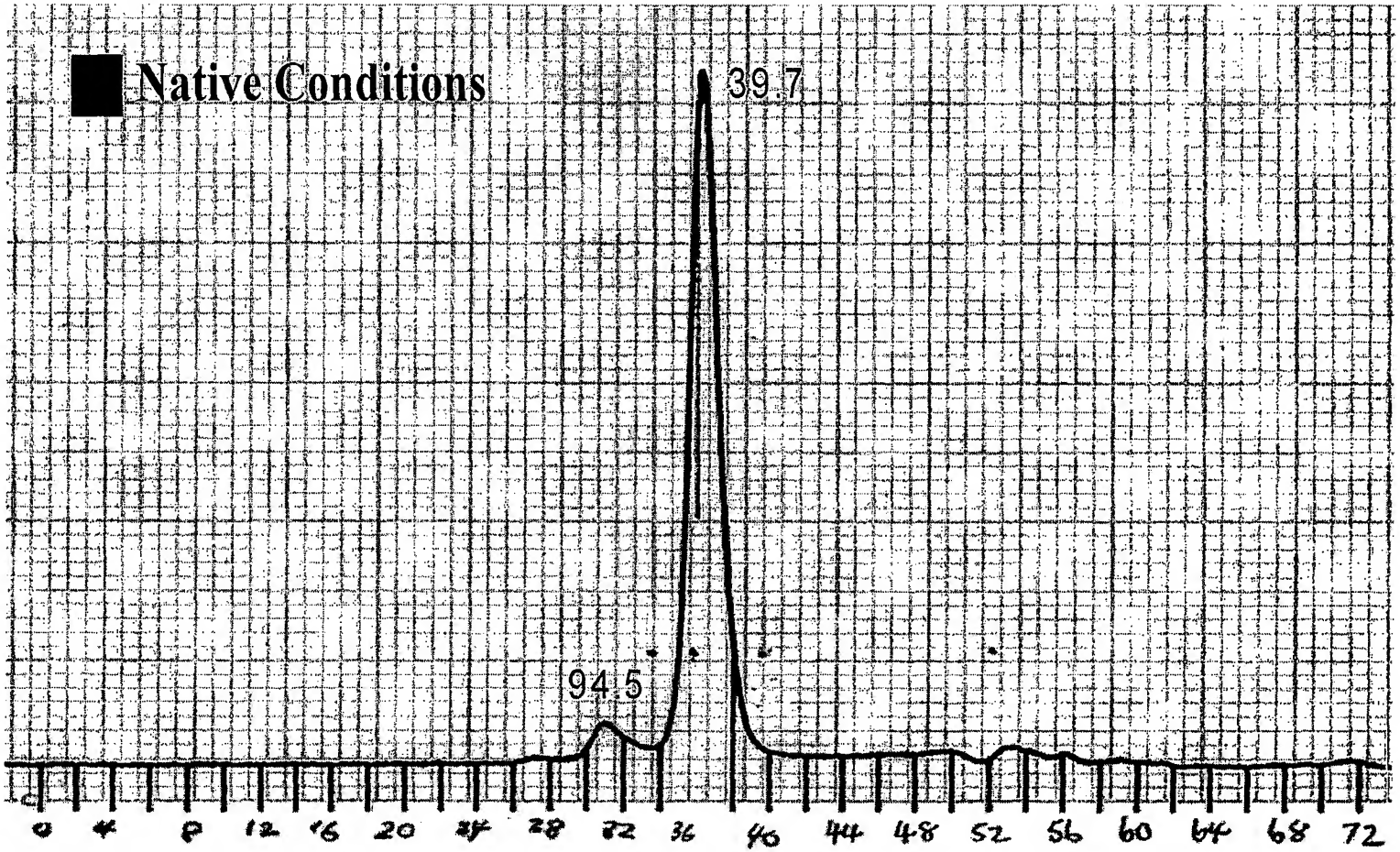

Native Conditions
39.7
94.5
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
68
72

FIGURE 10

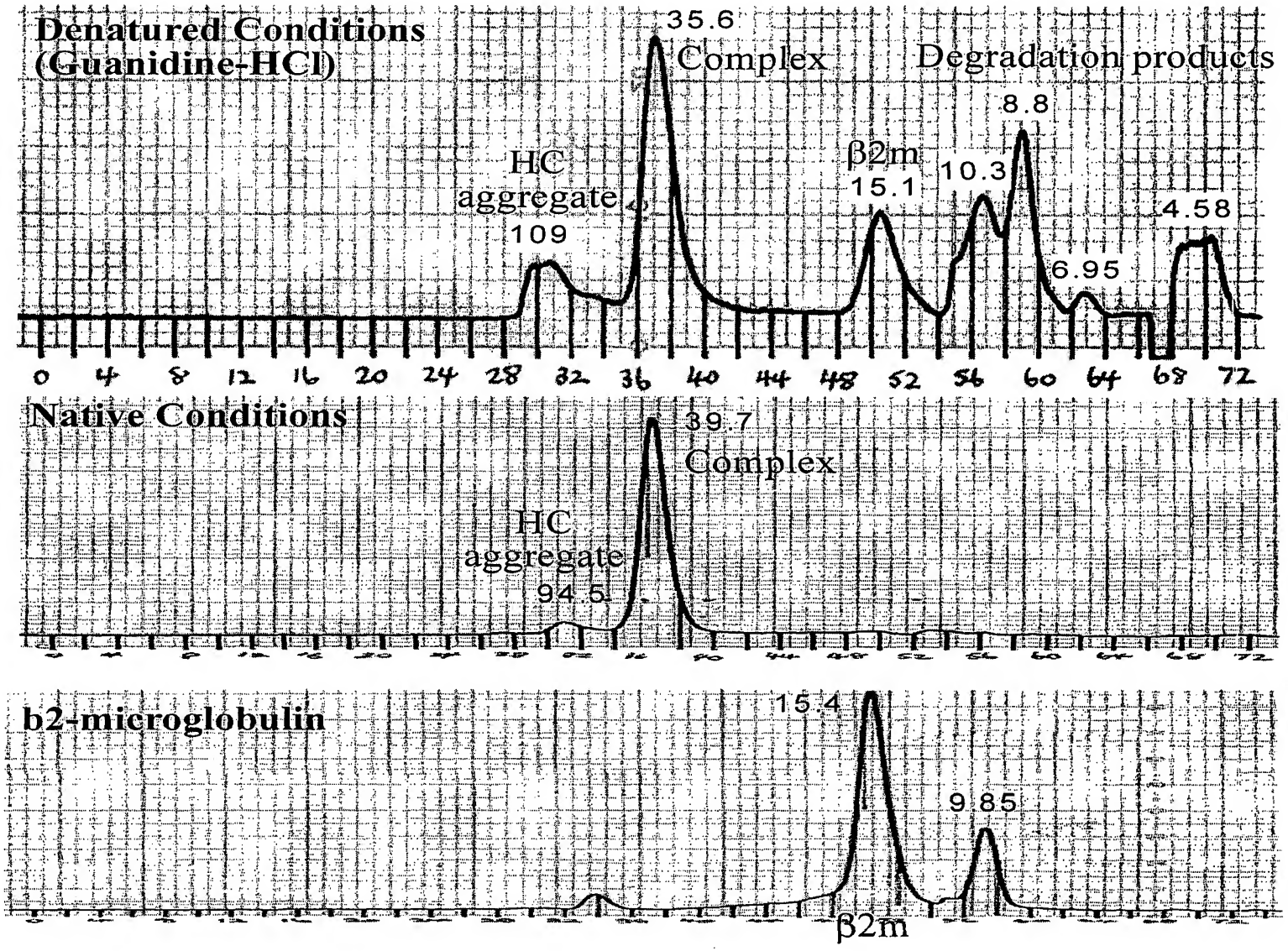

Denatured Conditions
(Guanidine-HCl)
35.6
Complex
Degradation products
8.8
β2m
15.1
10.3
HC
aggregate
109
4.58
6.95
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72
Native Conditions
39.7
Complex
HC
aggregate
94.5
b2-microglobulin
15.4
9.85
β2m

FIGURE 11

Coomassie-stained SDS-PAGE loaded with 2μg of protein

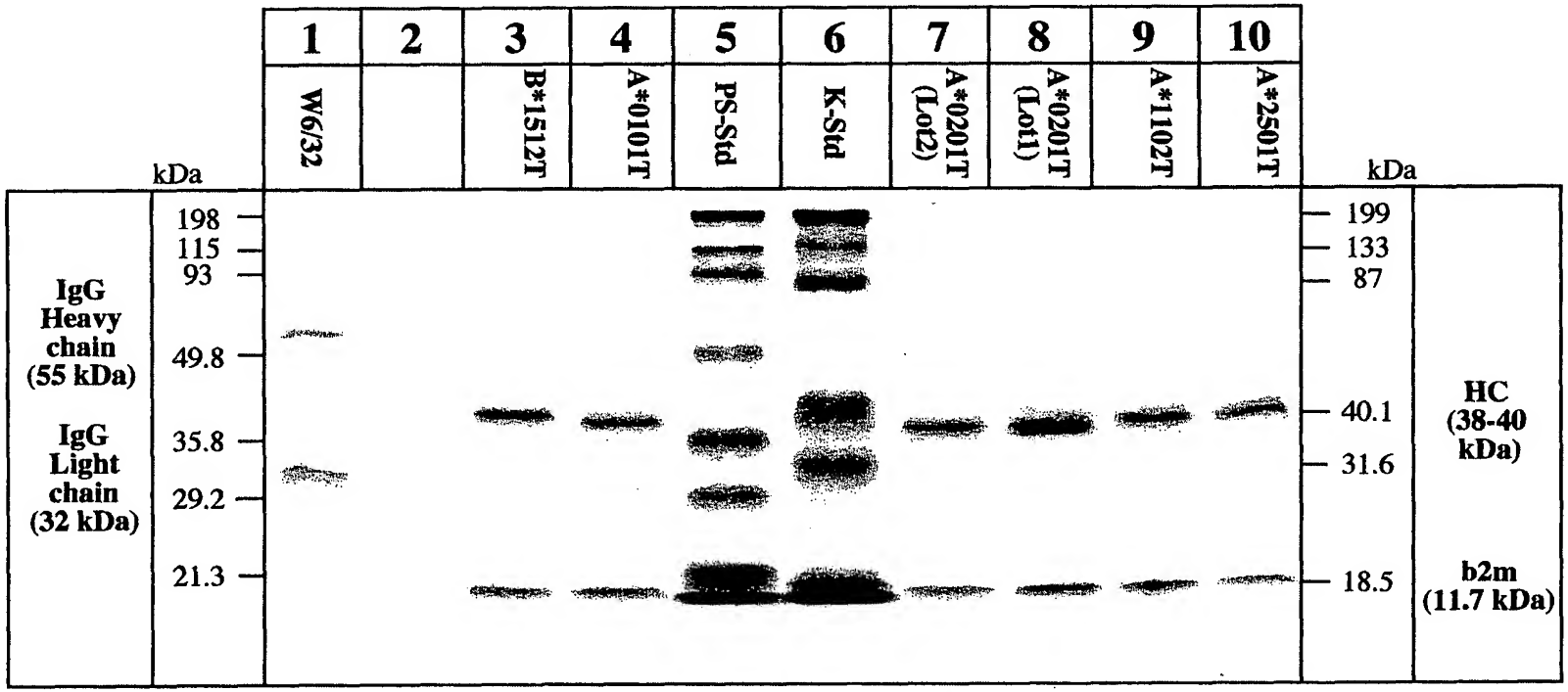


FIGURE 12

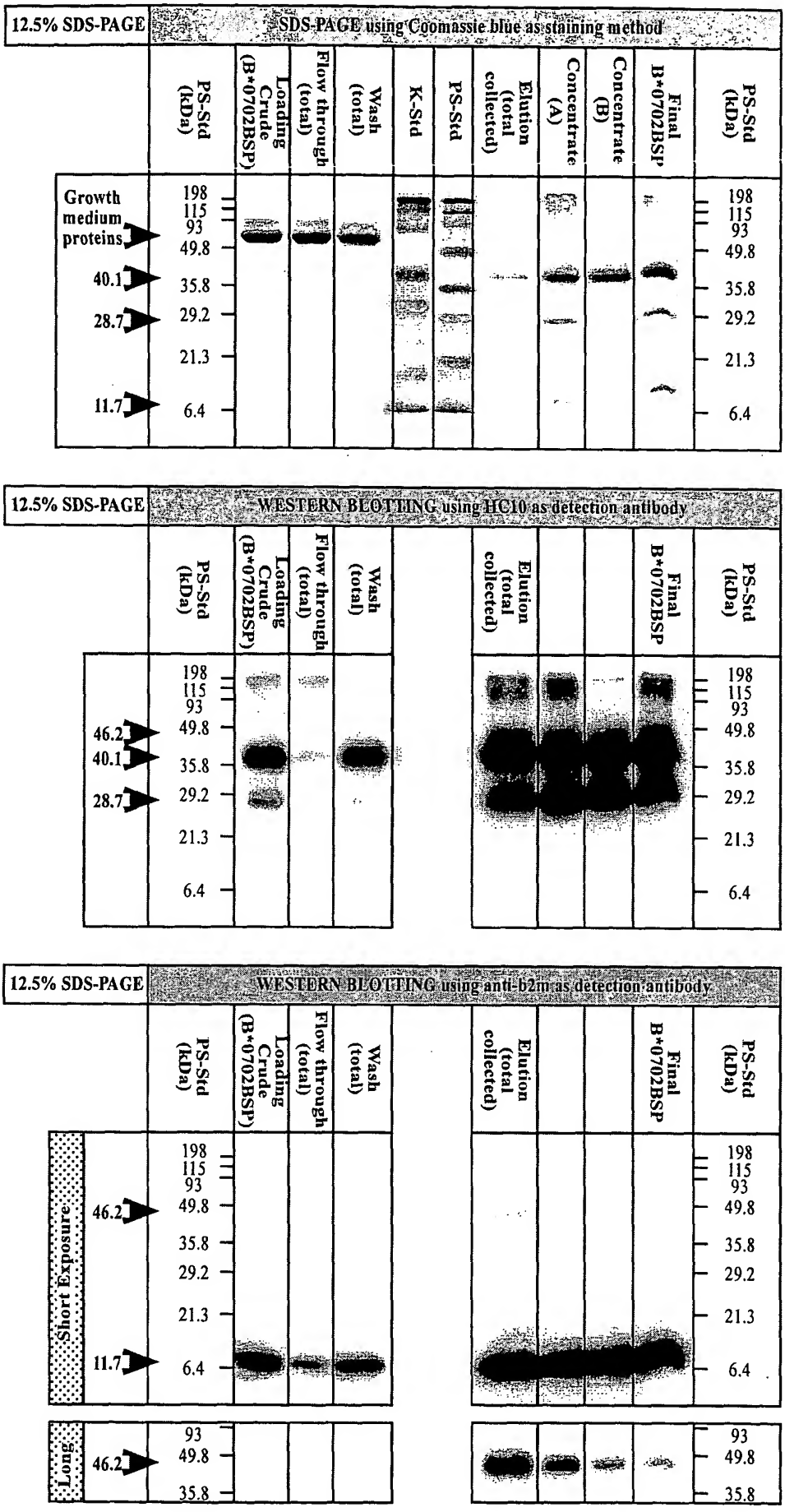

12.5% SDS-PAGE
SDS-PAGE using Coomassie blue as staining method
PS-Std (kDa)
Loading Crude (B*0702BSP)
Flow through (total)
Wash (total)
K-Std
PS-Std
Elution (total collected)
Concentrate (A)
Concentrate (B)
Final B*0702BSP
PS-Std (kDa)
Growth medium proteins
198
115
93
49.8
40.1
35.8
28.7
29.2
21.3
11.7
6.4
12.5% SDS-PAGE
WESTERN BLOTTING using HC10 as detection antibody
PS-Std (kDa)
Loading Crude (B*0702BSP)
Flow through (total)
Wash (total)
Elution (total collected)
Final B*0702BSP
PS-Std (kDa)
198
115
93
49.8
46.2
40.1
35.8
28.7
29.2
21.3
6.4
12.5% SDS-PAGE
WESTERN BLOTTING using anti-b2m as detection antibody
PS-Std (kDa)
Loading Crude (B*0702BSP)
Flow through (total)
Wash (total)
Elution (total collected)
Final B*0702BSP
PS-Std (kDa)
Short Exposure
198
115
93
49.8
46.2
35.8
29.2
21.3
11.7
6.4
Long
93
49.8
46.2
35.8

FIGURE 13

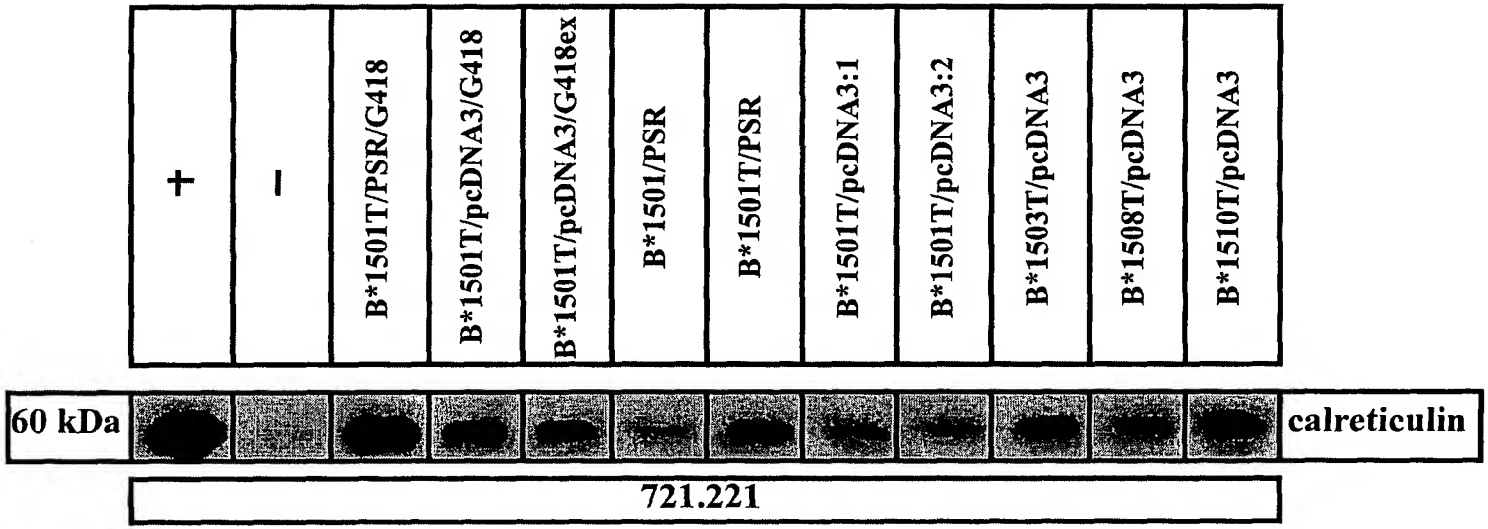

+
-
B*1501T/PSR/G418
B*1501T/pcDNA3/G418
B*1501T/pcDNA3/G418ex
B*1501/PSR
B*1501T/PSR
B*1501T/pcDNA3:1
B*1501T/pcDNA3:2
B*1503T/pcDNA3
B*1508T/pcDNA3
B*1510T/pcDNA3
60 kDa
calreticulin
721.221

FIGURE 14

B*1501 Peptide-binding specificities

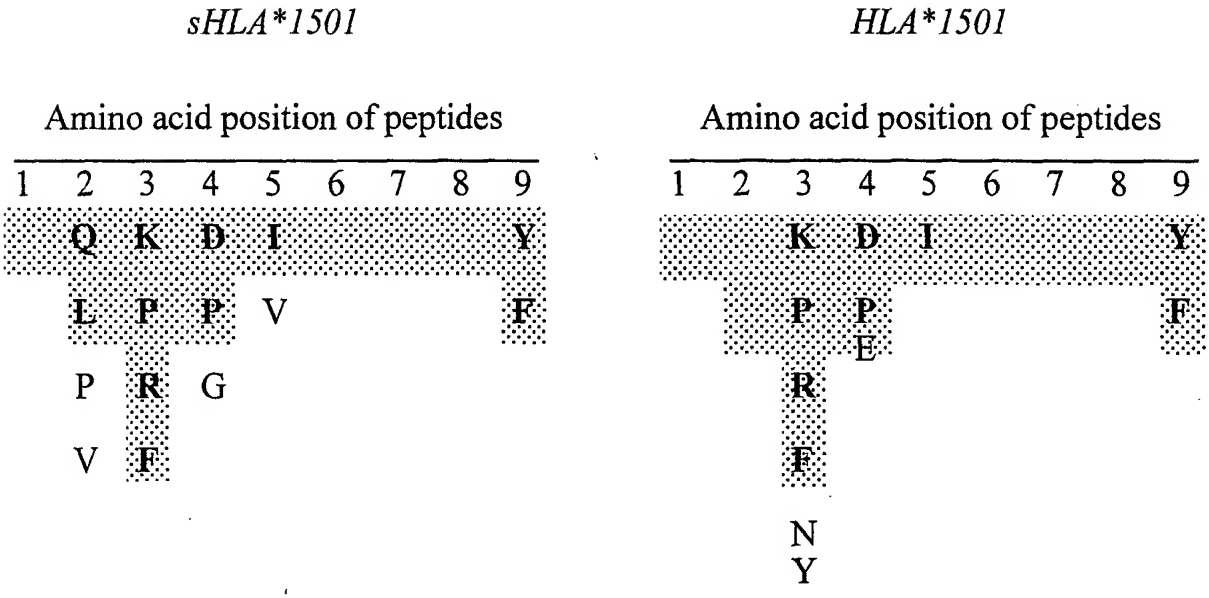
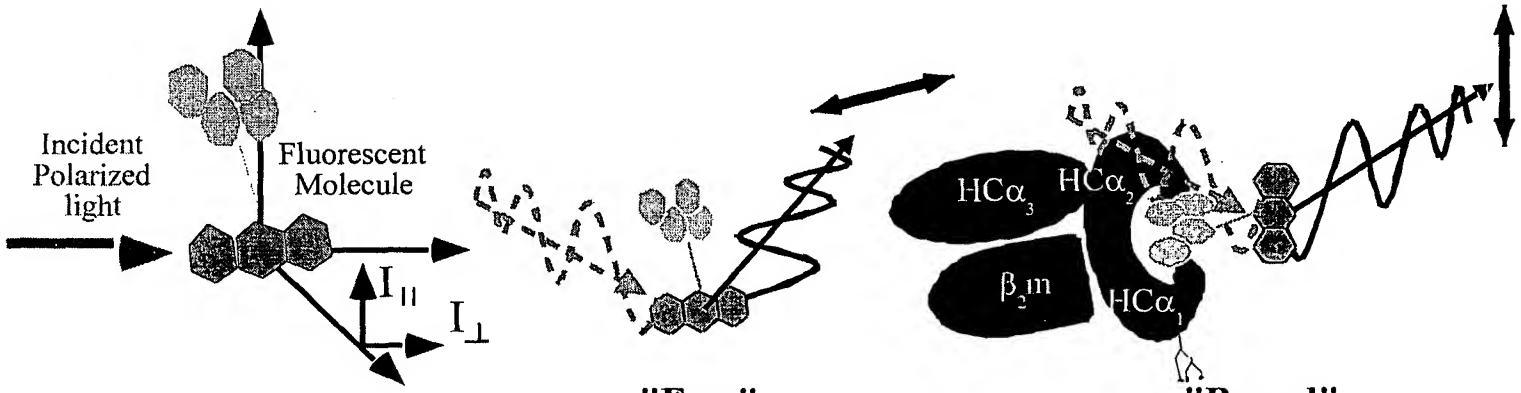


FIGURE 15

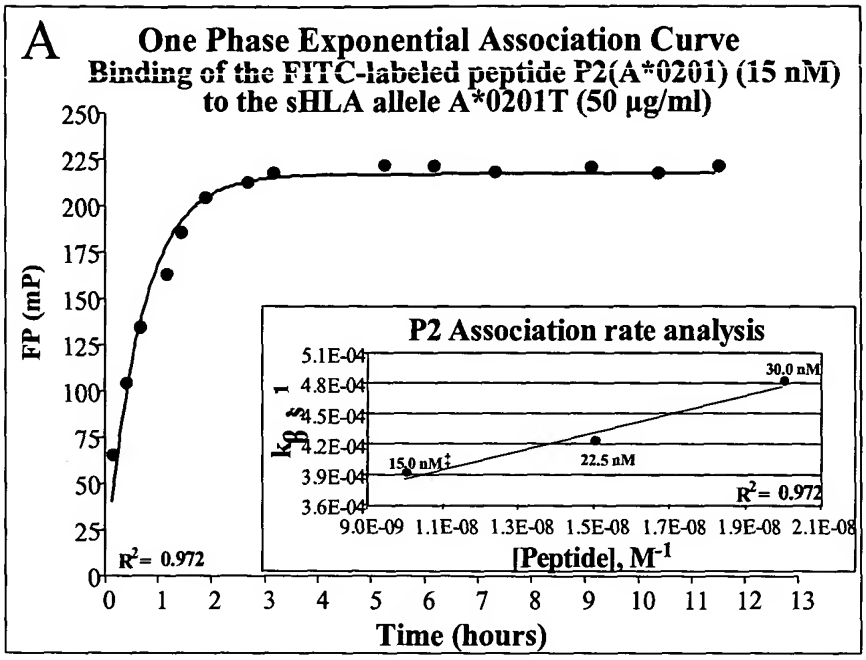


Parallel and Perpendicular

"Free" fluorescent ligand Low FP

"Bound" fluorescent ligand High FP

FIGURE 16


A
One Phase Exponential Association Curve
Binding of the FITC-labeled peptide P2(A*0201) (15 nM) to the sHLA allele A*0201T (50 µg/ml)
FP (mP)
Time (hours)
R²= 0.972
P2 Association rate analysis
30.0 nM
22.5 nM
15.0 nM
R²= 0.972
[Peptide], M⁻¹

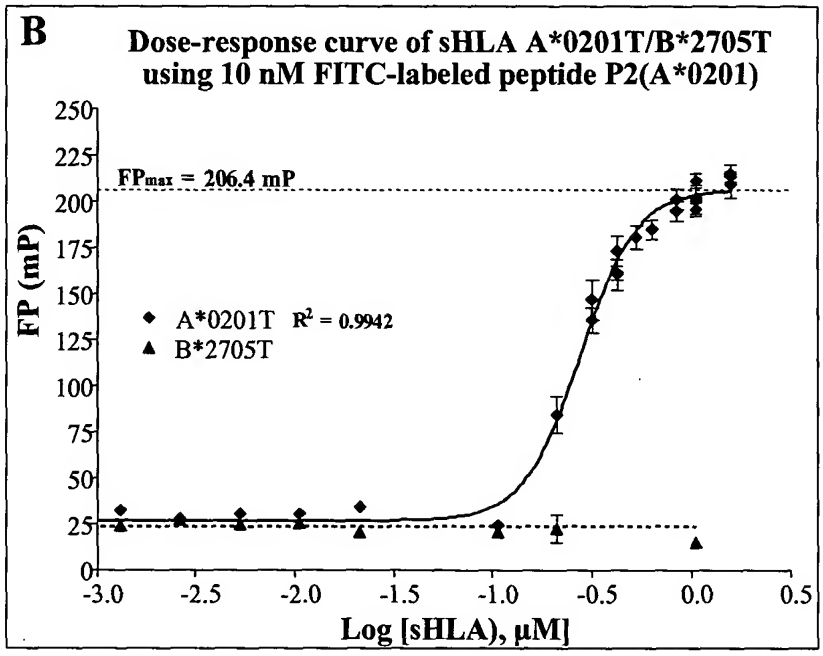
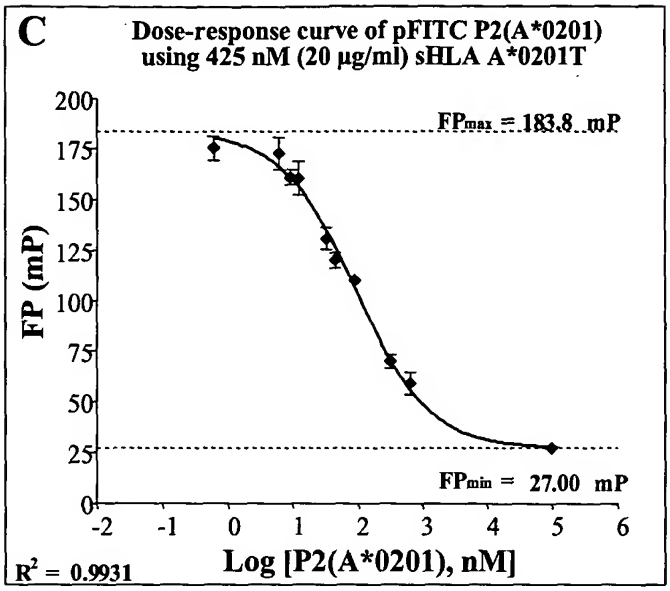

B
Dose-response curve of sHLA A*0201T/B*2705T using 10 nM FITC-labeled peptide P2(A*0201)
FPmax = 206.4 mP
A*0201T R² = 0.9942
B*2705T
FP (mP)
Log [sHLA), µM]

FIGURE 17



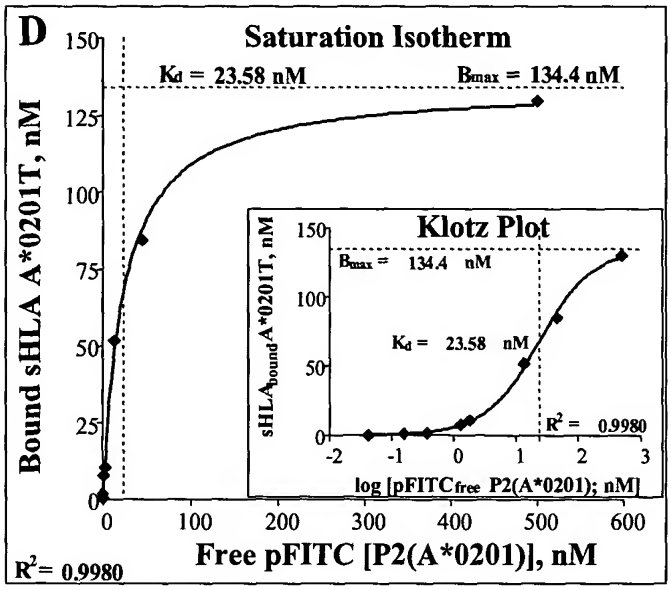


FIGURE 17 CONT'D

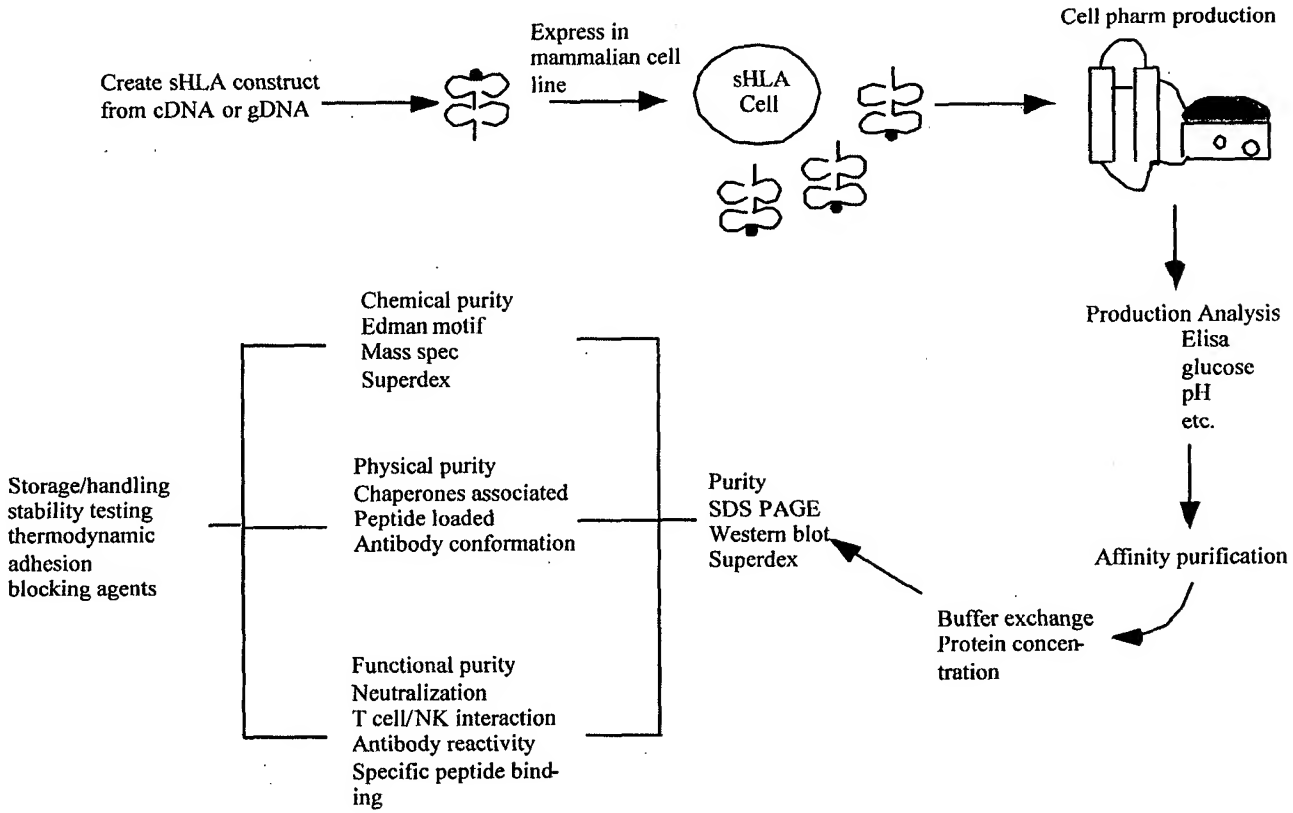
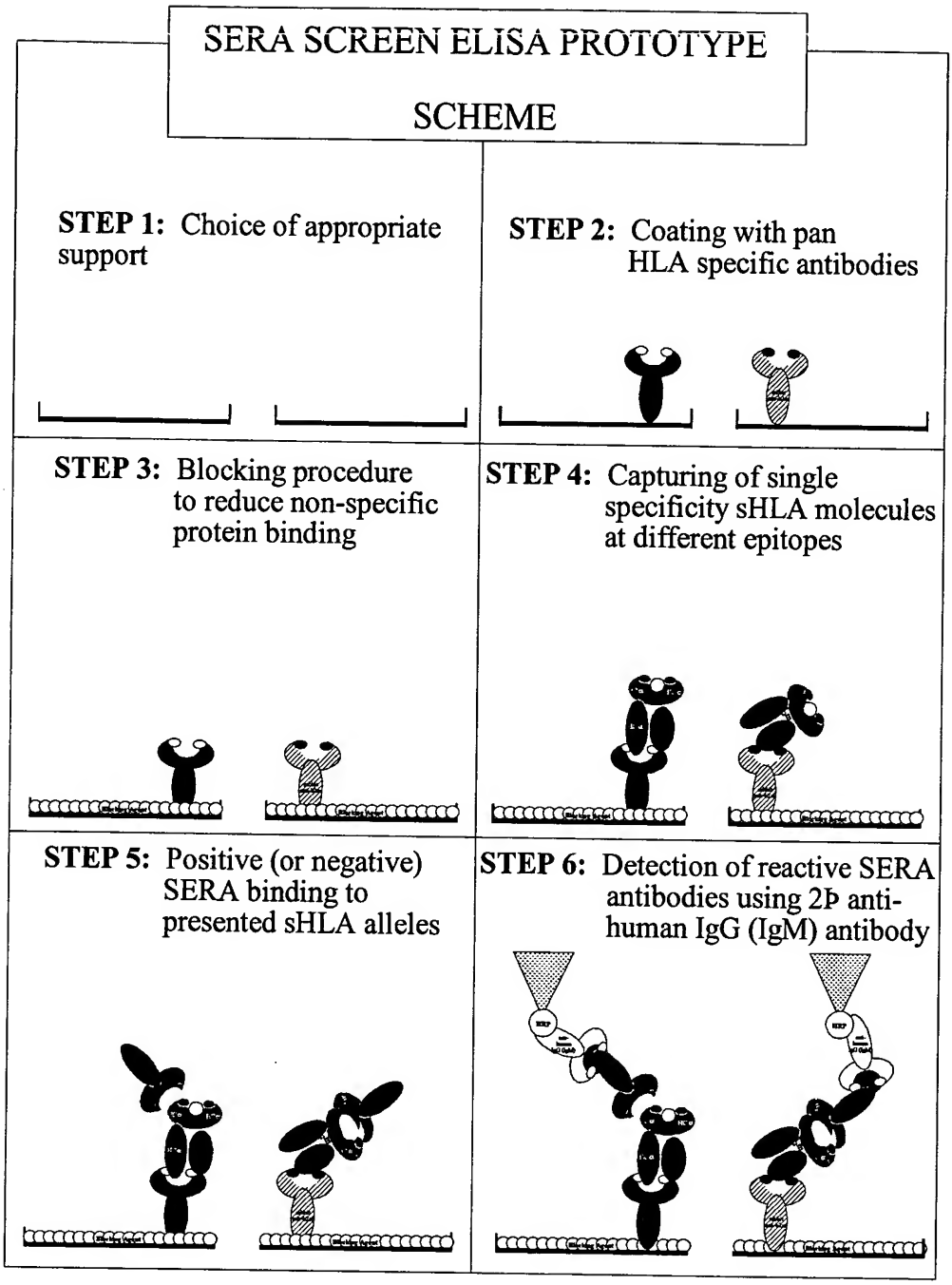

Create sHLA construct from cDNA or gDNA
Express in mammalian cell line
sHLA Cell
Cell pharm production
Production Analysis
Elisa
glucose
pH
etc.
Affinity purification
Buffer exchange
Protein concentration
Purity
SDS PAGE
Western blot
Superdex
Chemical purity
Edman motif
Mass spec
Superdex
Physical purity
Chaperones associated
Peptide loaded
Antibody conformation
Functional purity
Neutralization
T cell/NK interaction
Antibody reactivity
Specific peptide binding
Storage/handling
stability testing
thermodynamic
adhesion
blocking agents

FIGURE 18

FIGURE 19



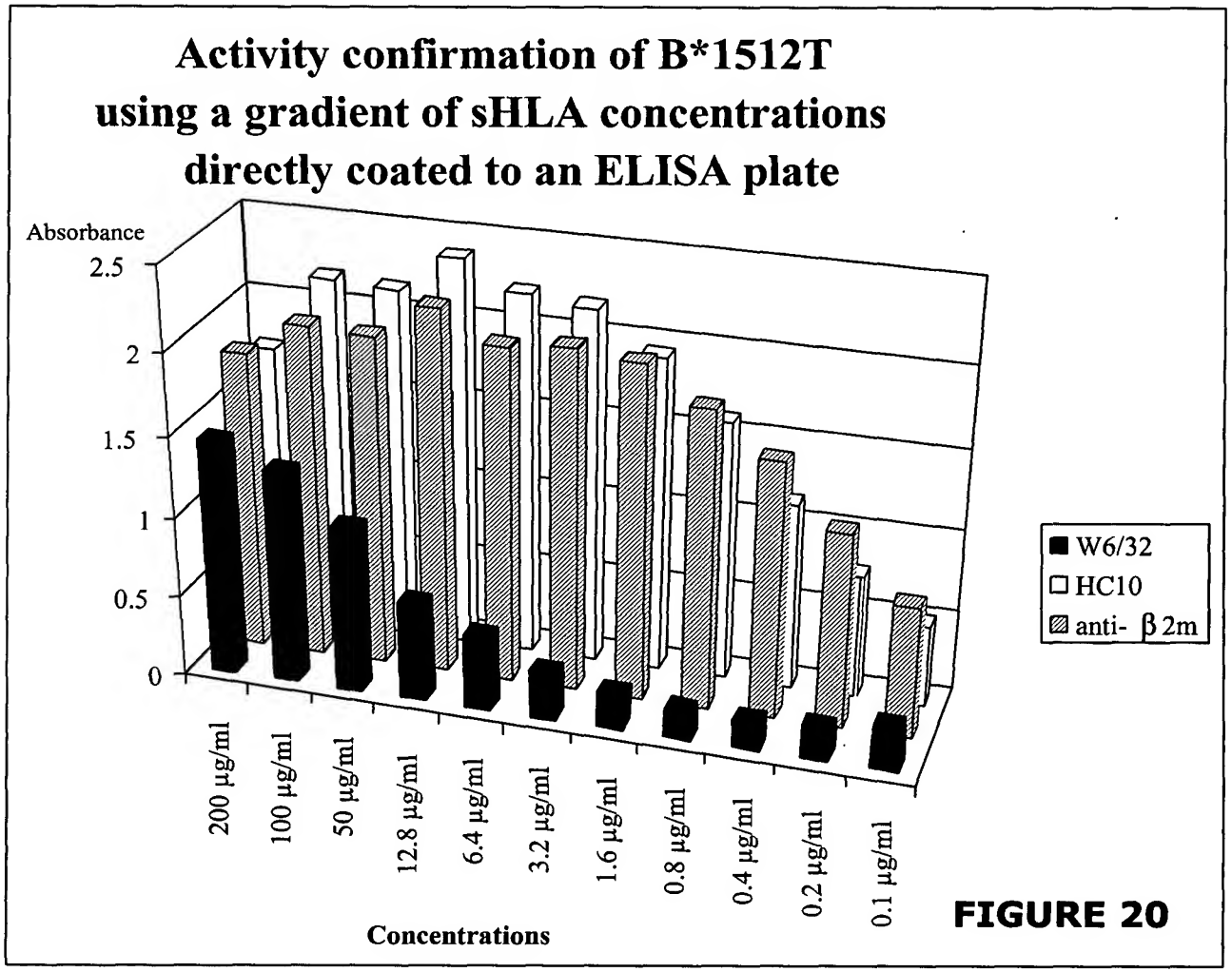

Activity confirmation of B*1512T
using a gradient of sHLA concentrations
directly coated to an ELISA plate
Absorbance
2.5
2
1.5
1
0.5
0
200 µg/ml
100 µg/ml
50 µg/ml
12.8 µg/ml
6.4 µg/ml
3.2 µg/ml
1.6 µg/ml
0.8 µg/ml
0.4 µg/ml
0.2 µg/ml
0.1 µg/ml
Concentrations
W6/32
HC10
anti- β2m

FIGURE 20

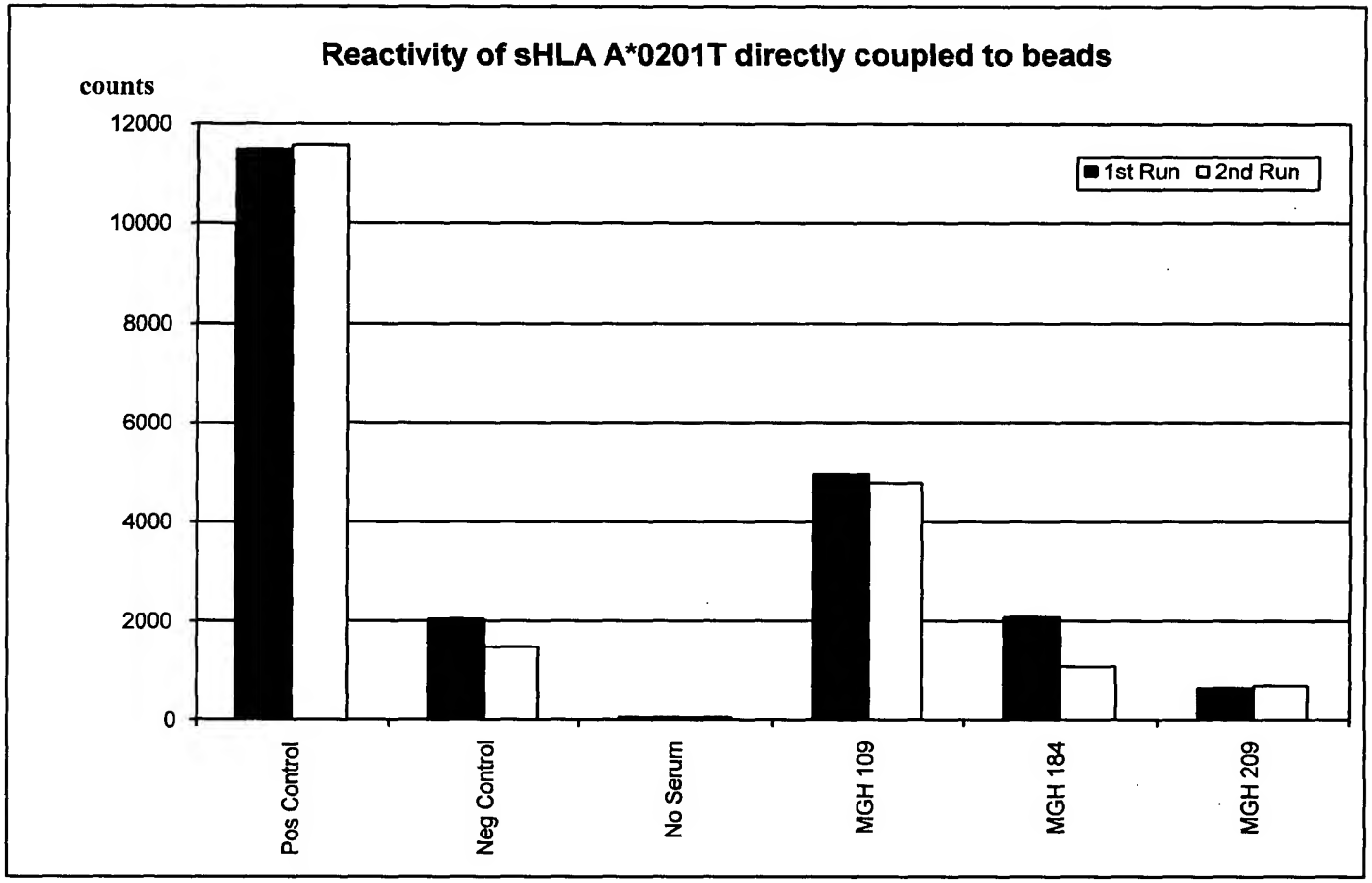

Reactivity of sHLA A*0201T directly coupled to beads
counts
12000
10000
8000
6000
4000
2000
0
1st Run
2nd Run
Pos Control
Neg Control
No Serum
MGH 109
MGH 184
MGH 209

FIGURE 21

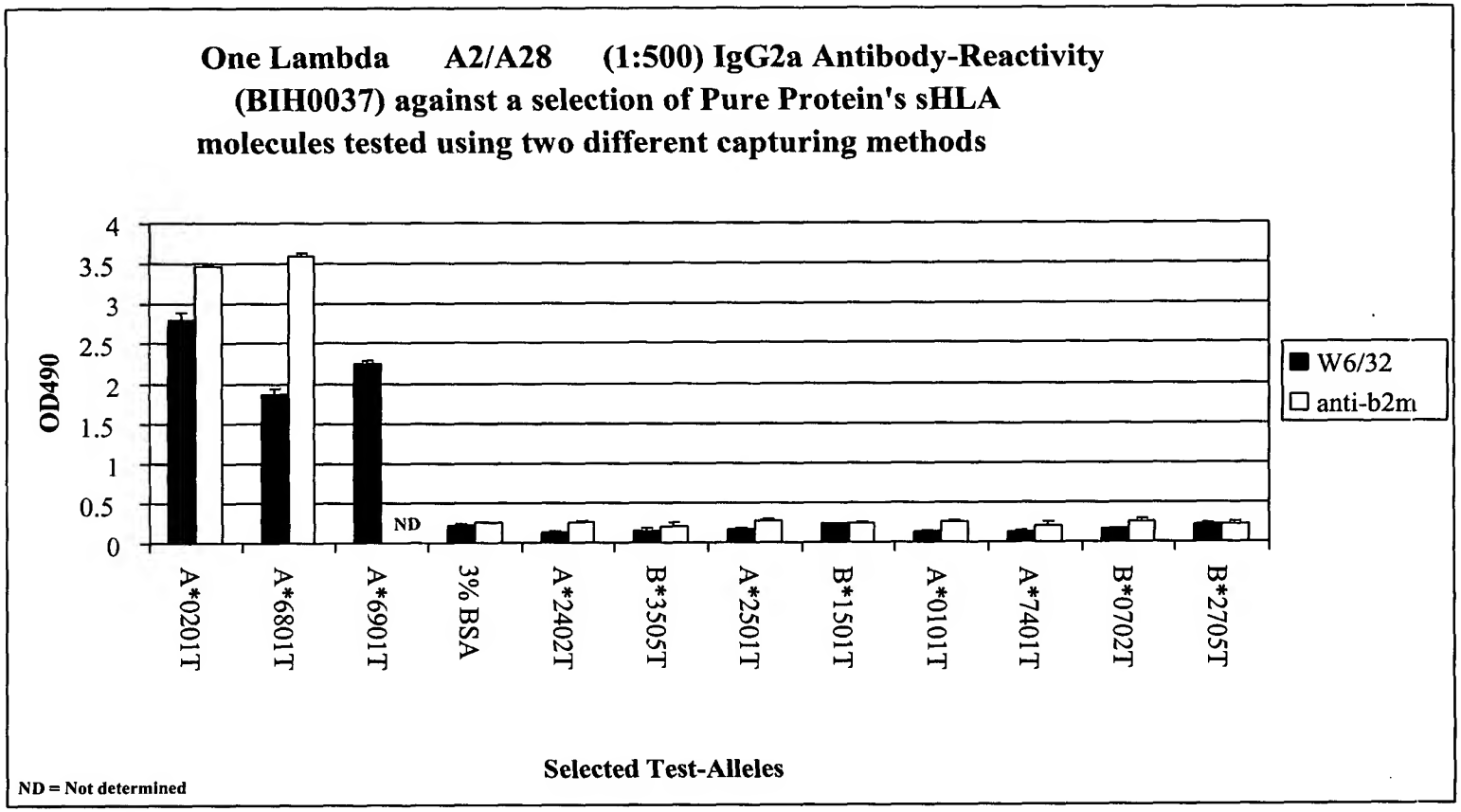

One Lambda A2/A28 (1:500) IgG2a Antibody-Reactivity (BIH0037) against a selection of Pure Protein's sHLA molecules tested using two different capturing methods
OD490
4
3.5
3
2.5
2
1.5
1
0.5
0
ND
A*0201T
A*6801T
A*6901T
3% BSA
A*2402T
B*3505T
A*2501T
B*1501T
A*0101T
A*7401T
B*0702T
B*2705T
W6/32
anti-b2m
Selected Test-Alleles
ND = Not determined

FIGURE 22

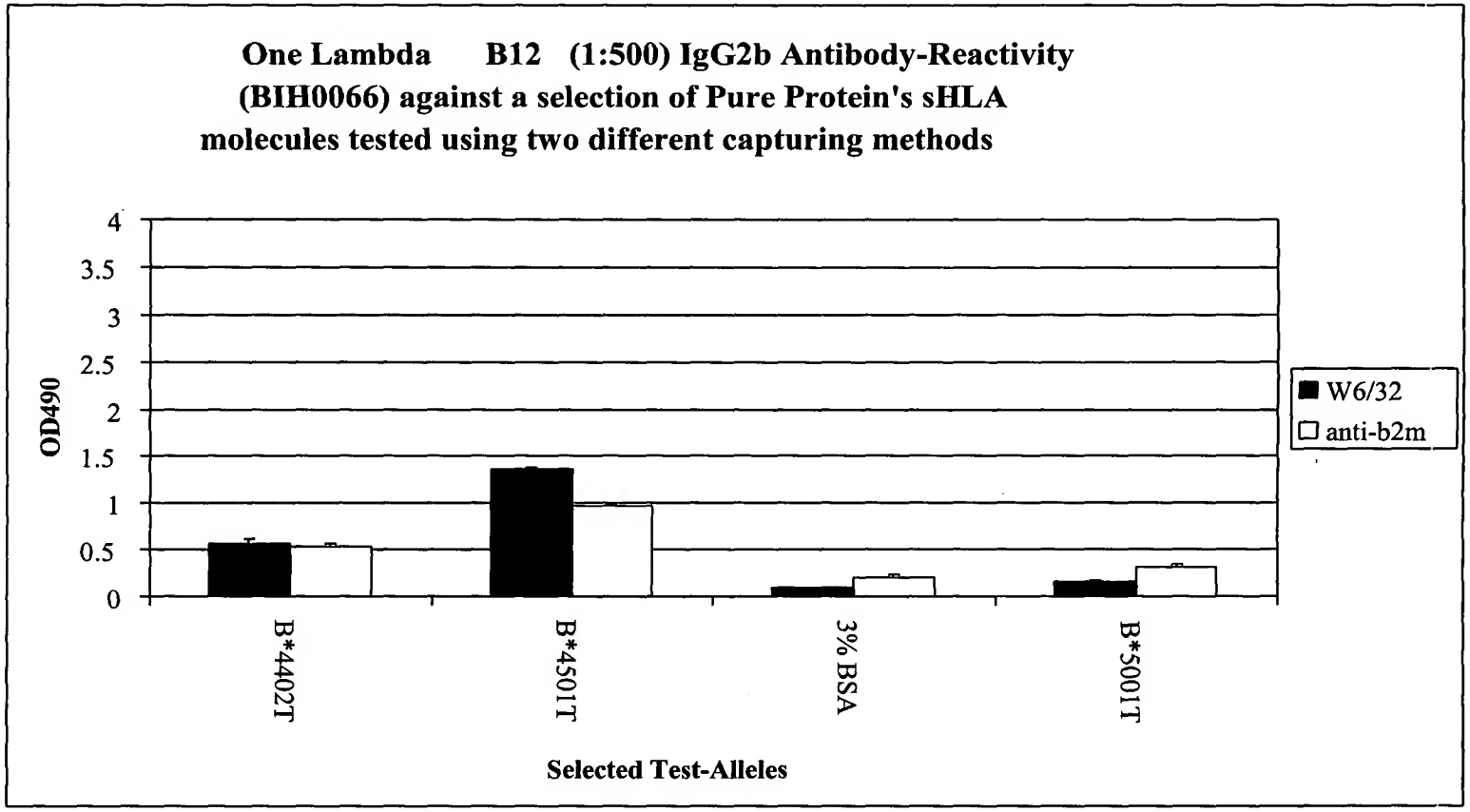

One Lambda B12 (1:500) IgG2b Antibody-Reactivity (BIH0066) against a selection of Pure Protein's sHLA molecules tested using two different capturing methods
OD490
4
3.5
3
2.5
2
1.5
1
0.5
0
B*4402T
B*4501T
3% BSA
B*5001T
Selected Test-Alleles
W6/32
anti-b2m

FIGURE 23

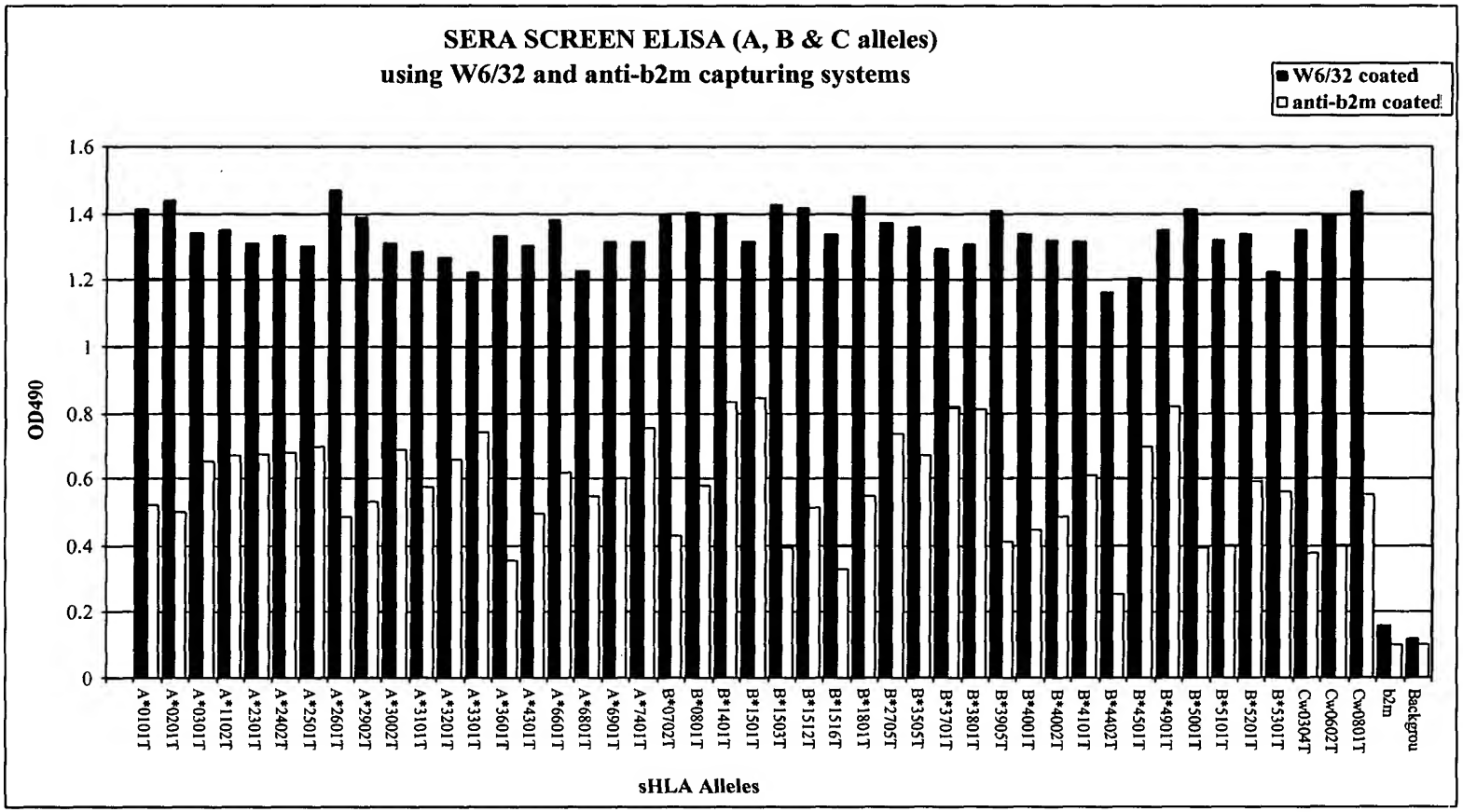

SERA SCREEN ELISA (A, B & C alleles)
using W6/32 and anti-b2m capturing systems
W6/32 coated
anti-b2m coated
OD490
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
A*0101T
A*0201T
A*0301T
A*1102T
A*2301T
A*2402T
A*2501T
A*2601T
A*2902T
A*3002T
A*3101T
A*3201T
A*3301T
A*3601T
A*4301T
A*6601T
A*6801T
A*6901T
A*7401T
B*0702T
B*0801T
B*1401T
B*1501T
B*1503T
B*1512T
B*1516T
B*1801T
B*2705T
B*3505T
B*3701T
B*3801T
B*3905T
B*4001T
B*4002T
B*4101T
B*4402T
B*4501T
B*4901T
B*5001T
B*5101T
B*5201T
B*5301T
Cw0304T
Cw0602T
Cw0801T
b2m
Backgrou
sHLA Alleles

FIGURE 24

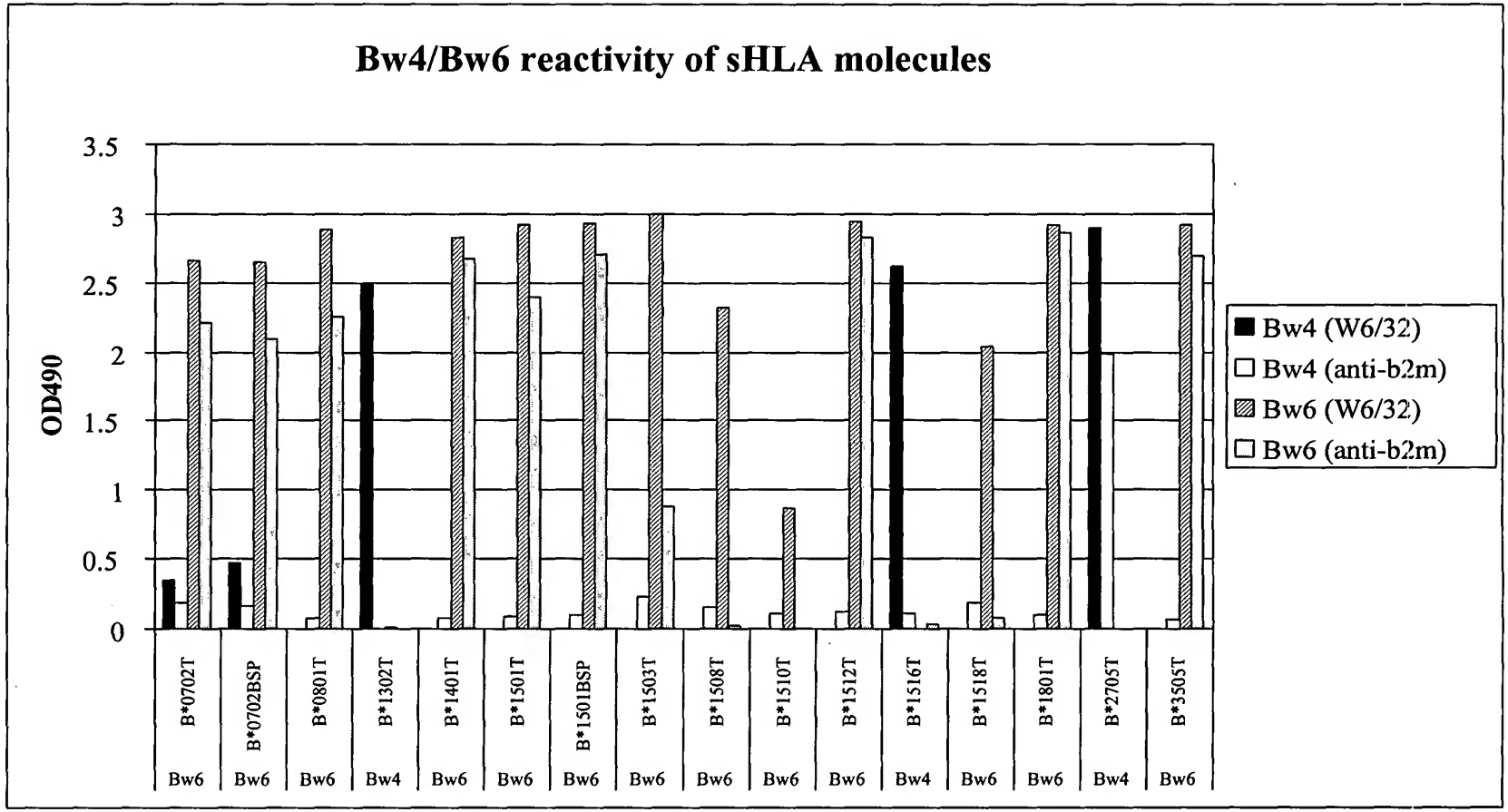

Bw4/Bw6 reactivity of sHLA molecules
OD490
3.5
3
2.5
2
1.5
1
0.5
0
Bw4 (W6/32)
Bw4 (anti-b2m)
Bw6 (W6/32)
Bw6 (anti-b2m)
B*0702T
B*0702BSP
B*0801T
B*1302T
B*1401T
B*1501T
B*1501BSP
B*1503T
B*1508T
B*1510T
B*1512T
B*1516T
B*1518T
B*1801T
B*2705T
B*3505T
Bw6
Bw6
Bw6
Bw4
Bw6
Bw6
Bw6
Bw6
Bw6
Bw6
Bw6
Bw4
Bw6
Bw6
Bw4
Bw6

FIGURE 25

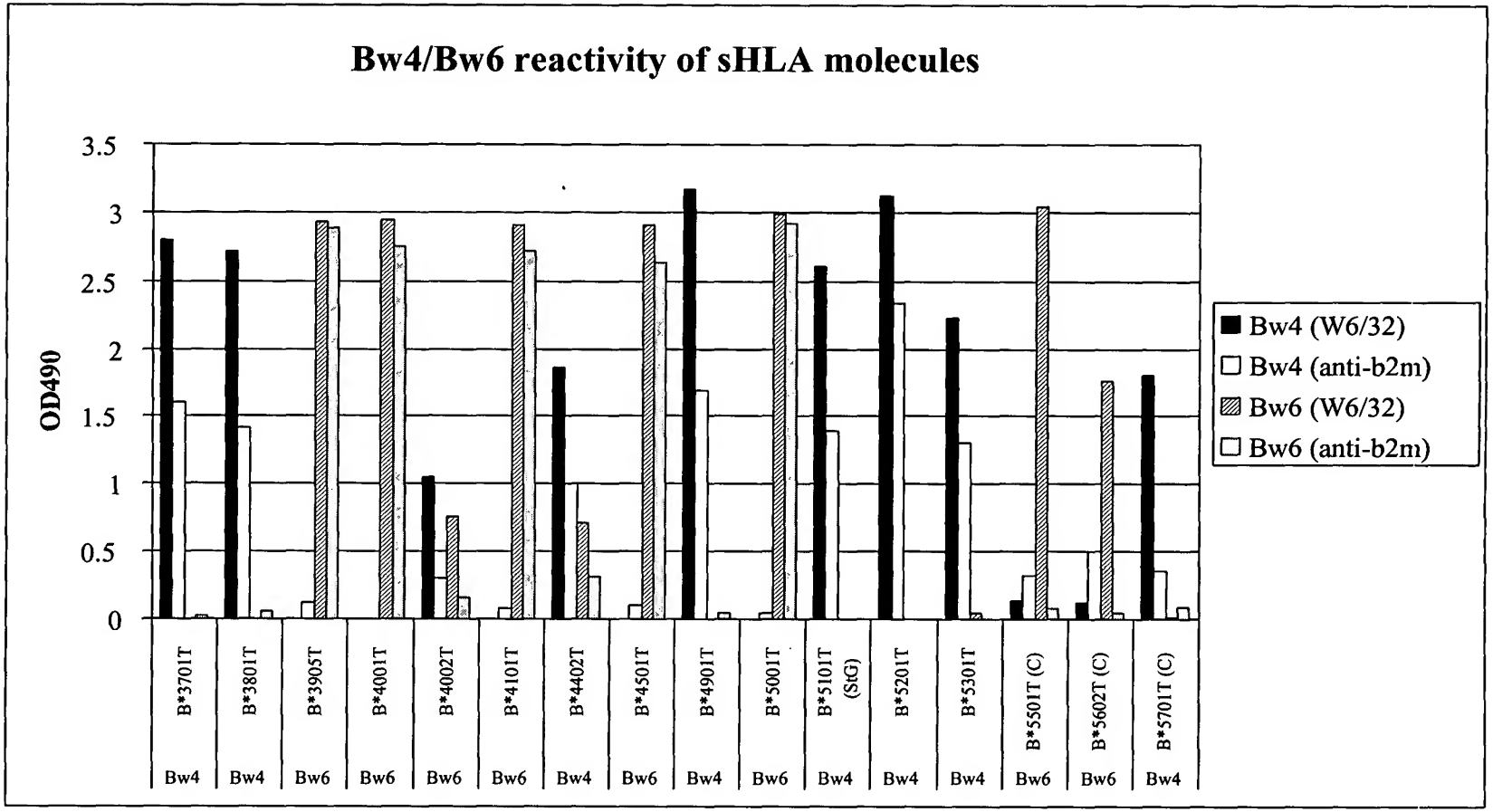

Bw4/Bw6 reactivity of sHLA molecules
OD490
3.5
3
2.5
2
1.5
1
0.5
0
Bw4 (W6/32)
Bw4 (anti-b2m)
Bw6 (W6/32)
Bw6 (anti-b2m)
B*3701T Bw4
B*3801T Bw4
B*3905T Bw6
B*4001T Bw6
B*4002T Bw6
B*4101T Bw6
B*4402T Bw4
B*4501T Bw6
B*4901T Bw4
B*5001T Bw6
B*5101T (StG) Bw4
B*5201T Bw4
B*5301T Bw4
B*5501T (C) Bw6
B*5602T (C) Bw6
B*5701T (C) Bw4

FIGURE 26

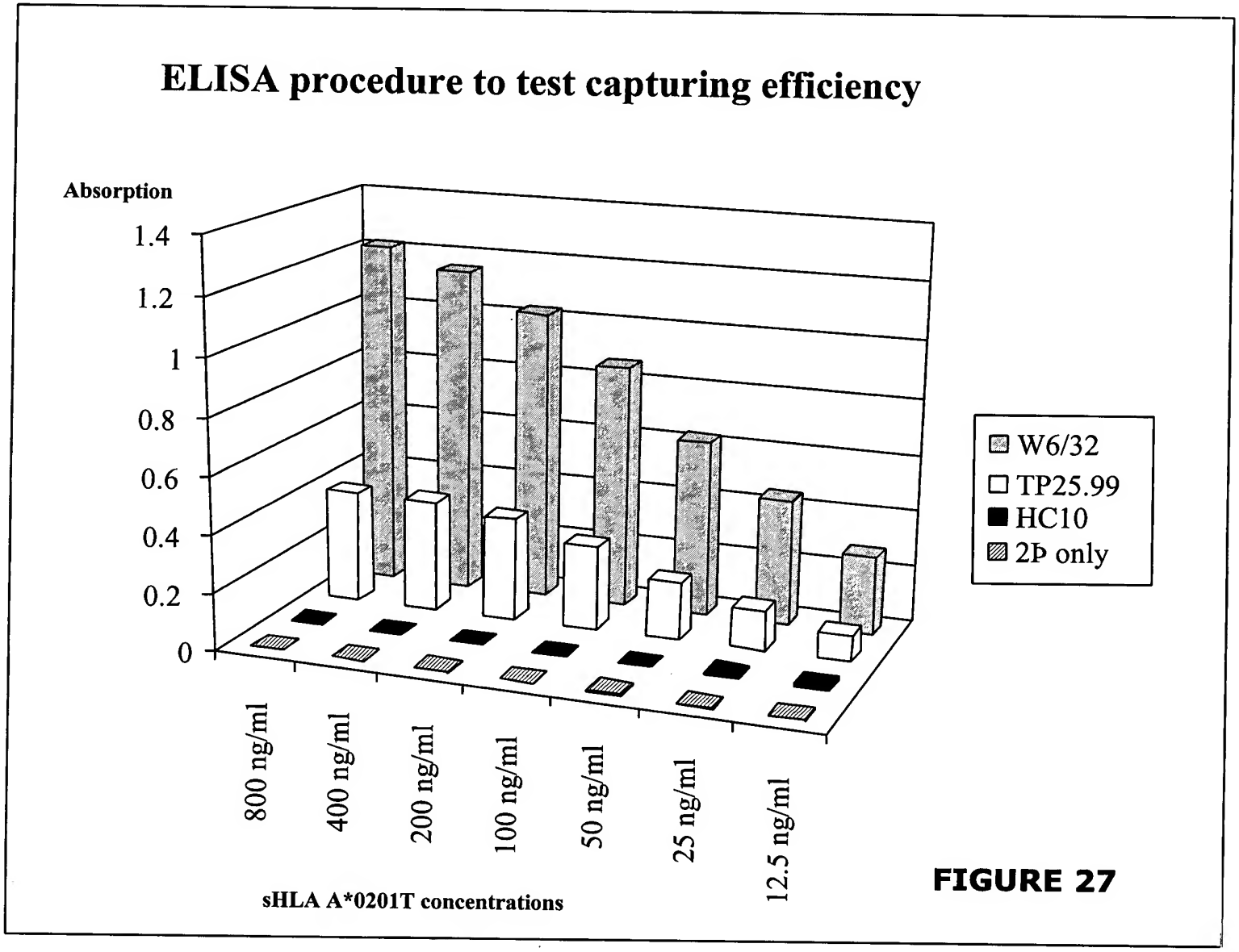

ELISA procedure to test capturing efficiency
Absorption
1.4
1.2
1
0.8
0.6
0.4
0.2
0
800 ng/ml
400 ng/ml
200 ng/ml
100 ng/ml
50 ng/ml
25 ng/ml
12.5 ng/ml
sHLA A*0201T concentrations
W6/32
TP25.99
HC10
2þ only

FIGURE 27

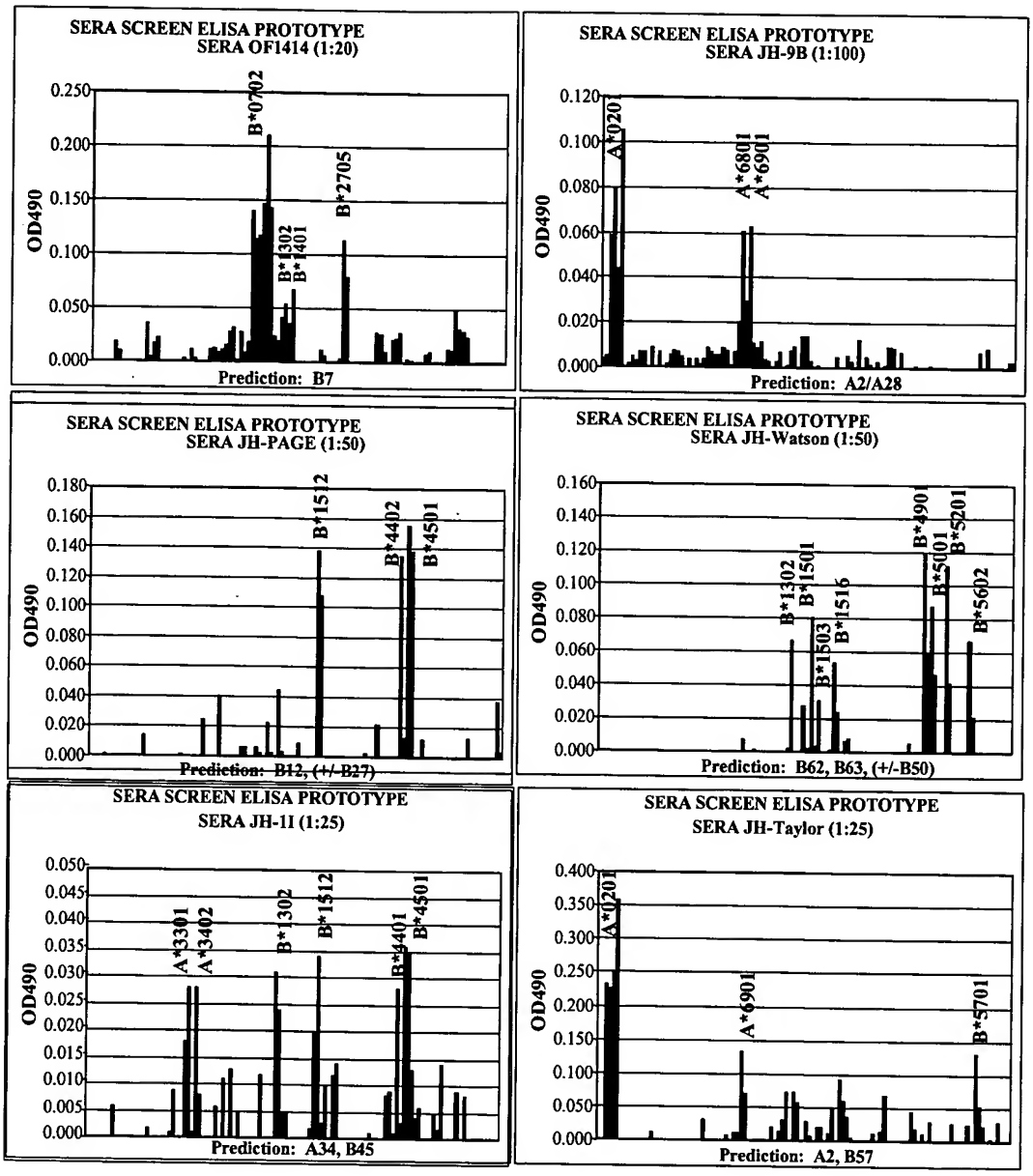

SERA SCREEN ELISA PROTOTYPE
SERA OF1414 (1:20)
OD490
B*0702
B*2705
B*1302
B*1401
Prediction: B7
SERA SCREEN ELISA PROTOTYPE
SERA JH-9B (1:100)
OD490
A*0201
A*6801
A*6901
Prediction: A2/A28
SERA SCREEN ELISA PROTOTYPE
SERA JH-PAGE (1:50)
OD490
B*1512
B*4402
B*4501
Prediction: B12, (+/-B27)
SERA SCREEN ELISA PROTOTYPE
SERA JH-Watson (1:50)
OD490
B*1302
B*1501
B*1503
B*1516
B*4901
B*5001
B*5201
B*5602
Prediction: B62, B63, (+/-B50)
SERA SCREEN ELISA PROTOTYPE
SERA JH-1I (1:25)
OD490
A*3301
A*3402
B*1302
B*1512
B*4401
B*4501
Prediction: A34, B45
SERA SCREEN ELISA PROTOTYPE
SERA JH-Taylor (1:25)
OD490
A*0201
A*6901
B*5701
Prediction: A2, B57

FIGURE 28

Positive and negative serum reaction against the A*0201T allele after background subtraction detected using the sandwich ELISA technique with W6/32 as capturing Ab and anti-human IgG(HRP) [Sigma A0170 / Made in goat (1:10'000)] as 2Þ antibody

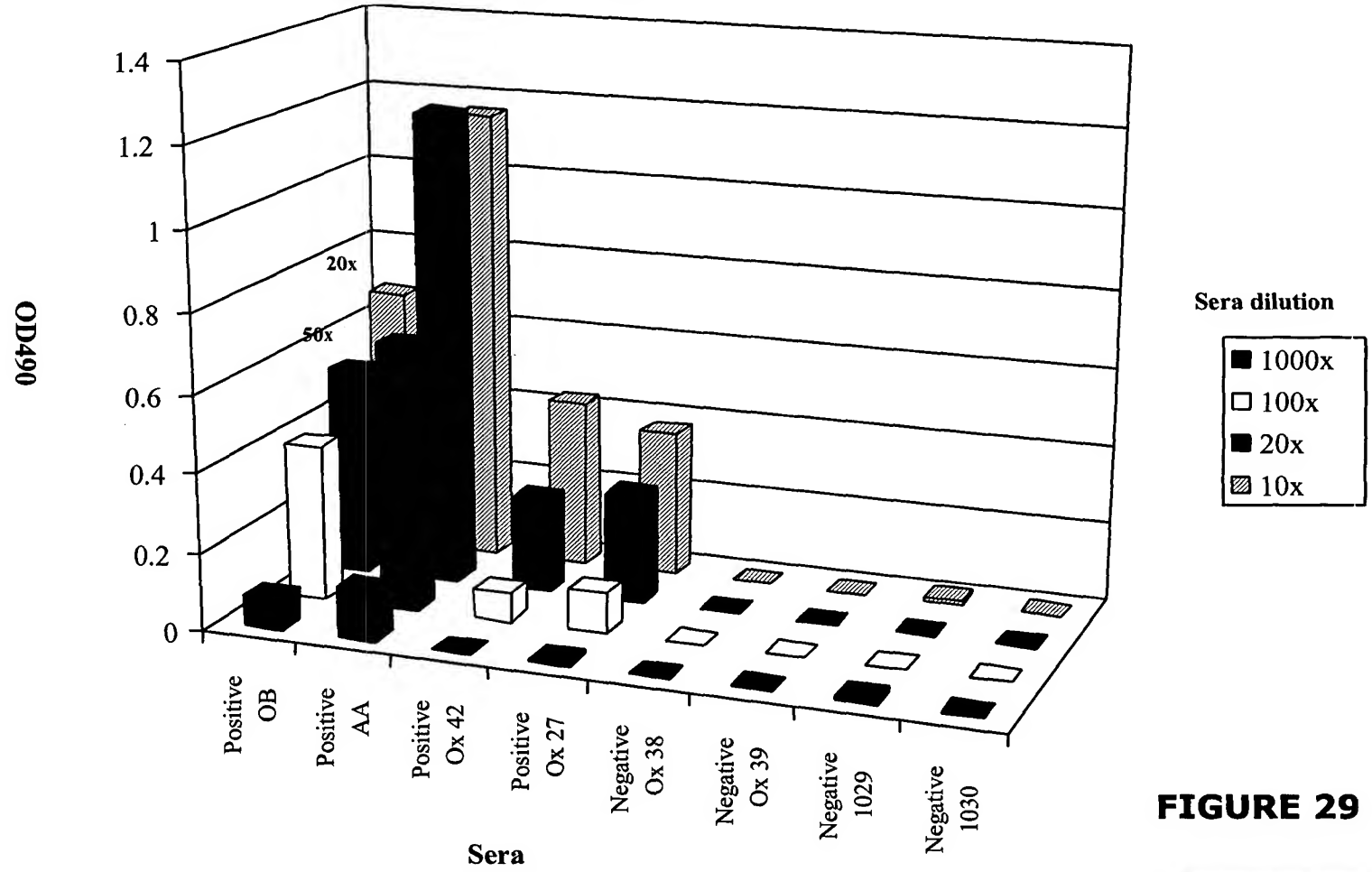


FIGURE 29